1974

Studies in heterogeneous catalytic exchange of some halogenated aromatic compounds, applying a mass spectrometer computer-system for isotope ratio determinations

Gary Richard Pallister
Wollongong University College

STUDIES IN HETEROGENEOUS CATALYTIC EXCHANGE OF SOME HALOGENATED AROMATIC COMPOUNDS, APPLYING A MASS SPECTROMETER COMPUTER-SYSTEM FOR ISOTOPE RATIO DETERMINATIONS

A thesis submitted in partial
fulfilment of the requirements
for the degree of

BACHELOR OF SCIENCE
with Honours

by

GARY RICHARD PALLISTER

Supervisor - Mr. W. K. Hannan

Wollongong University College,
The University of New South Wales.

November, 1974.

808076

SUMMARY

Some Group VIII metals, prepared by prereduction of their salts with sodium borohydride, were examined for catalytic activity with deuterium oxide and halogenated aromatic compounds in heterogeneous systems. Substantial exchange of the halobenzenes examined occurred on platinum and iridium, involving incorporation of up to three deuterium atoms per molecule. The other metals examined showed only a low level of deuterium incorporation for exchange of the halobenzenes. Extensive exchange of all ring protons, however, occurred with fluorobenzene on palladium. For exchange of ring halogenated toluenes, platinum was again generally active. Nickel favoured exchange of the methyl protons of these systems. Extensive exchange of ring and methyl protons of the fluorotoluenes occurred on palladium.

The results of the exchange of the chlorine and bromine substitued aromatics examined are consistent with "severe" ortho deactivation of ring protons adjacent to these groups. With fluorine the effect, whilst present, was much less pronounced. The exchange patterns observed are generally consistent with the theory of π-complex adsorption.

The existing Quadnova operating system was adapted to perform quantitative isotope ratio determinations. Each of the three methods developed showed an improvement in quantitative analyses compared with the orginal system. The method involving calculation of intensities by a peak area routine proved to be the most successful.

TABLE OF CONTENTS

INTRODUCTION

Interest in the preparation and use of stable isotopes has increased greatly in recent years[1,2]. This has not only been in chemistry but also in biology, medicine and allied areas. There are a number of factors which have promoted this increased interest in stable isotopes. Firstly advances in design and performance of magnetic and quadrupole mass spectrometers and nuclear magnetic resonance spectrometers have made the estimation of the enriched stable isotope content of a compound easier and more accurate. Secondly the increasing concern over the toxicity of radioisotopes, particularly for their use in medicine, has meant that the non-toxic, inert, stable isotopes are preferred. This is particularly so for their administration to infants and pregnant women in the detection of clinical abnormalities. Thirdly the economic cost of most enriched stable isotopes has decreased significantly in recent years.

One facet of stable isotope analysis with a mass spectrometer that has been explored in some detail recently is the direct determination of the total amount of isotopic enrichment in an organic molecule[3-6]. In favourable cases the position of enrichment can also be determined by this method. This has meant that the complex chemical degradative procedures of radioisotope technology are now usually unnecessary.

One of the many procedures for catalytic labelling (enrichment) of organic compounds that has received some attention in recent years is that of heterogeneous catalysis. A series of halogenated compounds

were chosen as a model for investigation. The method adopted involves a suitably prepared borohydride reduced[7,8] metal catalyst being mixed with a convenient source of deuterium (heavy water) and the organic substance to be exchanged. The exchange reaction is allowed to proceed in this heterogeneous system at elevated temperature in a sealed degassed ampoule. After an appropriate period of time the organic compound is removed from the ampoule and the deuterium content determined by a computer controlled quadrupole mass spectrometer, the Quadnova system.

A literature review of the published work concerning deuterium exchange of halogenated benzene derivatives was undertaken. Harper and Kemball[9] undertook a study of catalytic exchange and associated reactions with monohalogenated benzenes in gaseous systems. Deuterium gas was used as a source of deuterium and evaporated metal films as catalysts. The acid induced exchange of deuterium and tritium for hydrogen in bromobenzene is discussed by Olsson[10]. Weening in a review[11] discusses the use of exchange reactions to synthesise deuterated organic compounds. Tupitsyn and Zatsepina[12] examined the kinetics of base catalysed deuterium exchange of a large number of aromatic fluorine compounds including fluorobenzene and some fluorinated toluenes. Fraser and Renaud[13] determined the relative rates of platinum catalysed exchange of heavy water with the ortho, meta and para protons in several monosubstituted benzenes including the halobenzenes. Garnett[14,15] and Garnett and Sollich[16] discuss the exchange of heavy water and monohalogenated benzenes and napthalenes on

heterogeneous platinum catalysts at temperatures in the range 130-180^{o}C.

Direct analysis for deuterium content by mass spectrometry (especially with a quadrupole mass spectrometer) has been shown to be advantageous[1,5,17]. However the analyses performed using the Quadnova operating system (an integrated Gas Chromatograph - Mass Spectrometer - Computer System) were found to fluctuate between consecutive mass spectra when quantitative results were desired. Accordingly a number of changes were made to the software to produce a more quantitative system. Krypton and Xenon were used as reference gases of known isotopic distribution for evaluating the performance of the software.

DISCUSSION

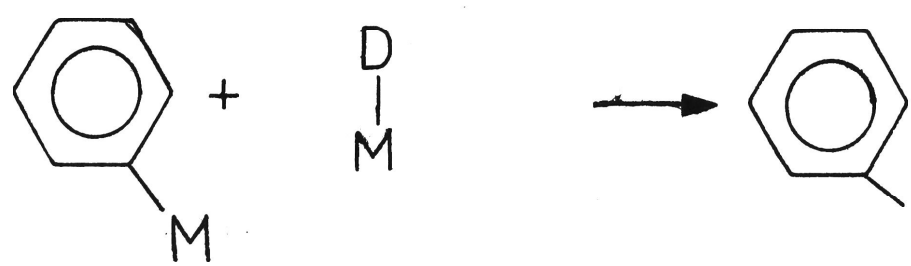
HETEROGENEOUS CATALYSIS

Classical and π-dissociative mechanisms

Horiuti and Polanyi[18] in 1933 and Farkas and Farkas[19] in 1934 performed the first deuterium exchange reactions. Mechanistic work done up till 1957 has been reviewed by Taylor[20]. The proposed classical mechanisms can be divided into associative and dissociative processes. In the Farkas and Farkas[19] dissociative mechanism the hydrogen chemisorbs through carbon hydrogen bond rupture (2). A deuteron then exchanges (3) with the chemisorbed molecule. This deuteron can come from either dissociatively adsorbed deuterium gas (1) or heavy water depending on which is used as a source of deuterium.

(1)

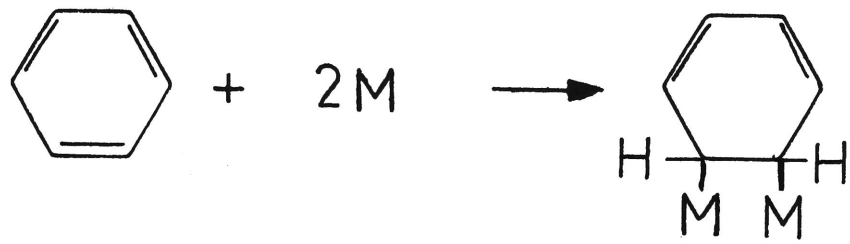
(2)



(3)

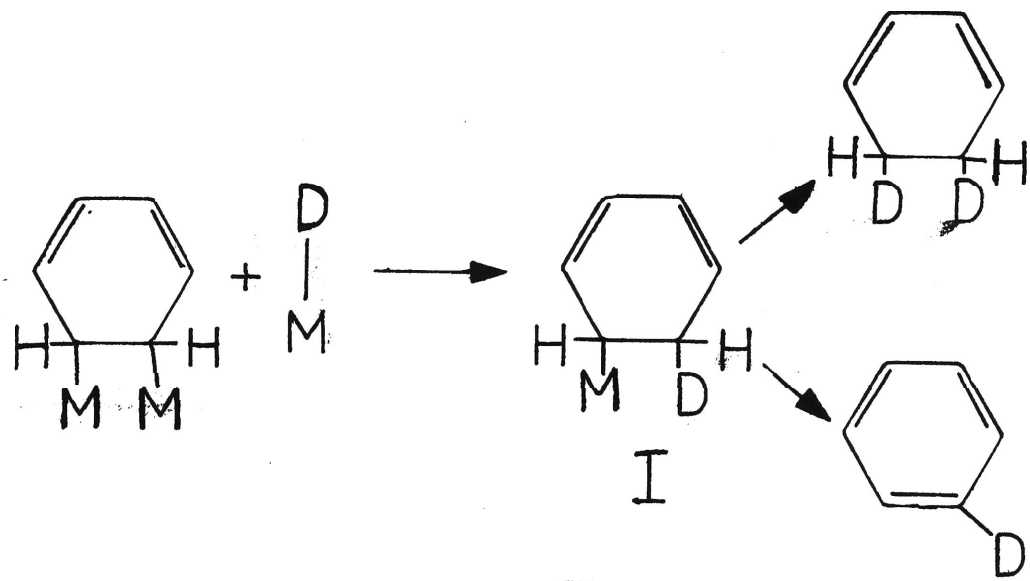
Farkas and Farkas postulate that hydrogenation and exchange result from two unrelated mechanisms. Hydrogenation is said to occur by the simultaneous addition of two chemisorbed hydrogen atoms to the physically adsorbed hydrocarbon in the Van der Waals layer.

Horiuti and Polanyi[18] in contrast in the classical associative mechanism postulate that both hydrogenation and exchange result from a common half hydrogenated state (I). The initial chemisorption of the unsaturated molecule is said to occur by opening of the double bond (4).



(4)

Exchange (6) or hydrogenation (5) then occurs through the half hydrogenated state (I).

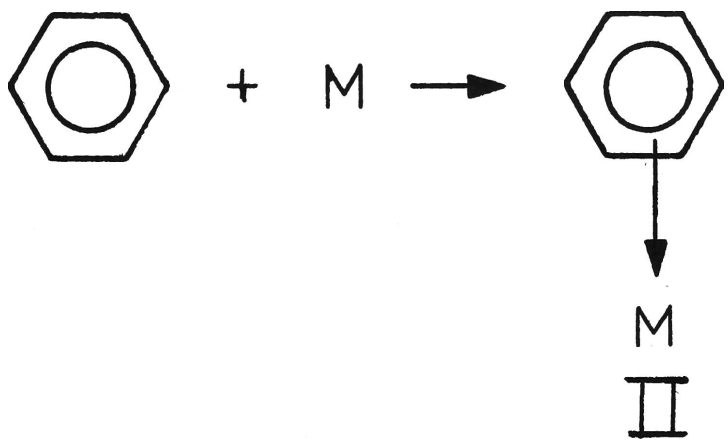


(5) Hydrogenation

Exchange (6)

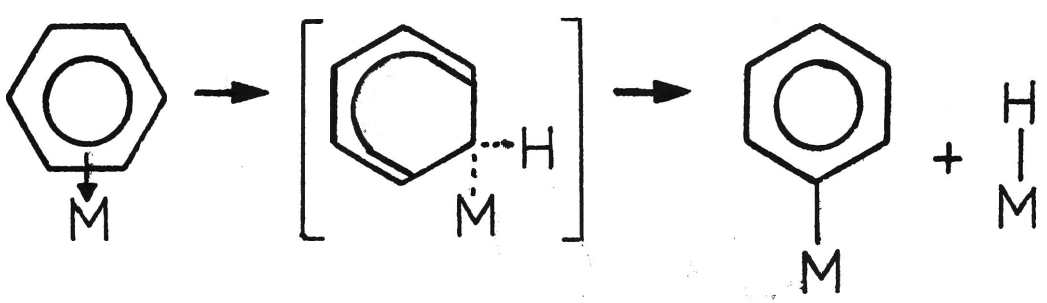
It is now generally accepted[15] that the dissociative mechanism applies to the exchange of saturated hydrocarbons. For unsaturated hydrocarbons and derivatives, however, there are a number of theories for the mechanism of exchange[20].

FIGURE 1.

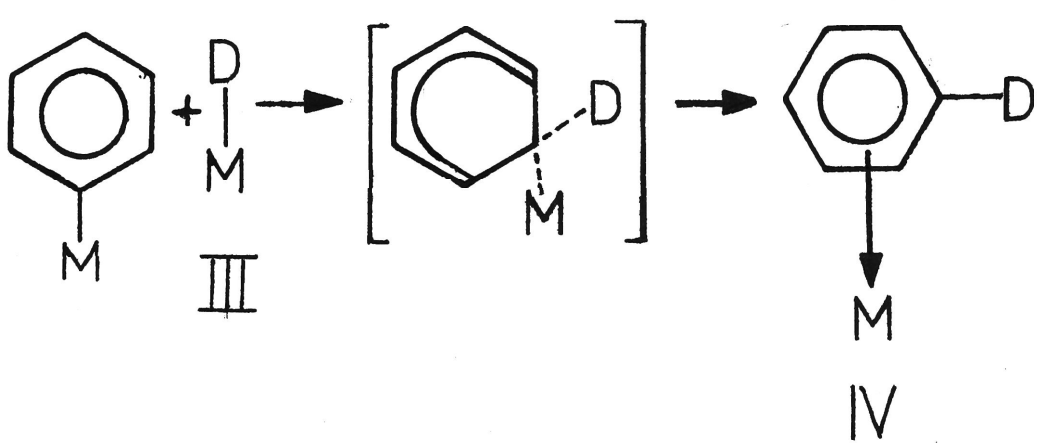


II

7.



8.



III

IV

9.

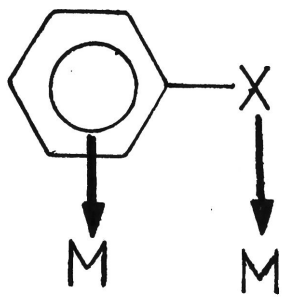
The π-complex dissociative mechanism is one current theory with general applicability for aromatic molecules. Garnett and co-workers[21] proposed this mechanism in 1960. π-bonded adsorption was also proposed by other workers including Bond[22], Crawford and Kemball[23] and Rooney[24] for different systems. Garnett[15, 21] states that the π-complex can be described in terms of Mullikens charge transfer theory[25] simply as a donor-acceptor interaction with the appropriate wave-function to describe the complex[26]. The important feature of the interaction is the net flow of charge, since π-complex adsorption involves the forward and back donation of electrons.

In essence π-complex intermediates involve the adsorption of the organic molecule on the surface of the metal by donation of π-electrons into the empty d-orbitals of the metal catalyst. There is also a significant donation of metal d-electrons into vacant anti-bonding orbitals of the organic molecule[27,28]. The net charge transfer is towards the metal as depicted in species (II).

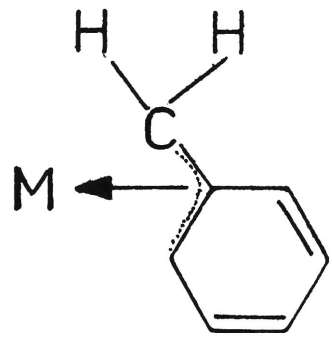
The adsorbed π-complexed aromatic (II) then reacts with an active site by a substitution process (8, 9). During this reaction the molecule rotates through 90° and changes from its horizontally π-complex adsorbed position to a vertically σ-bonded chemisorbed state. The postulated transition state for the π-σ bond conversion occurs when the plane of the rotating benzene molecule is approximately 45° to the catalyst surface. Rotation of the ring is necessary since "edge-on" π-complexing is prevented by orbital symmetry and by the steric hindrance of aromatic hydrogen atoms. The final step in the dissociative mechanism (figure 1) involves the σ-bonded aromatic undergoing a second, slower, substitution

reaction at the carbon-metal bond with a chemisorbed deuterium atom and then returning to the π-bonded state (reaction 9). The aromatic molecule after returning to the π-bonded state (species II) may then either desorb as a mono-deuterated compound or undergo further exchange.

The adsorption process described above may be modified if a polar substituent is present in the aromatic (species V); for example bromo-benzene.



V



VI

This polar substituent can lead to additional charge transfer interactions on the catalyst surface[15]. These interactions can then influence the nature of the exchange reaction by supplementing the π-electron adsorption.

Modified π-complex mechanisms have been proposed for the exchange of molecules which contain allylic protons such as the alkyl benzenes[15]. This modification involves the extension of the mechanism to involve π-allylic intermediates[23,24,29] of the type shown in (species VI). These intermediates predict enhanced exchange of the protons on a carbon atom to an aromatic ring, which is consistent with the reported observations[15].

Decreased exchange rates in the ortho positions[31,32] of alkyl benzenes have been observed and these have been attributed to steric effects as large and sterically crowded substituents (e.g. t-butyl) tend

to decrease the rate of exchange more than smaller substituents (e.g. methyl). Fraser and Renaud[13] have shown similar effects for protium exchange of highly deuterated chlorobenzene and bromobenzene using platinum catalyst. Brown and Garnett[33] have examined the exchange of bromobenzene and found that the reaction was slow. Although they do not make a specific comment on ortho deactivation, a suggestion of general deactivation of ortho positions is made. Garnett and Sollich[16] investigated the deuterium exchange of monosubstitued benzenes and found ortho deactivation for chlorine, bromine and iodine.

TABLE 1 DEUTERATION OF CHLOROBENZENE

CATALYST	TEMP. °C	Deuterium Incorporation (% Total)						% D *
		D_o	D_1	D_2	D_3	D_4	D_5	
Platinum	50	60.5	26.8	8.2	3.2	1.0	0.3	11.6
Platinum	110	18.5	19.9	21.6	31.2	7.4	1.5	38.7
Platinum	150	2.2	11.6	35.6	47.2	3.4	0	47.6
Palladium	50	99.6	0.4	0	0	0	0	0.2
Palladium	110	99.4	0.5	0.1	0	0	0	0.2
Palladium	150	99.1	0.6	0.3	0	0	0	0.3
Nickel	50	99.7	0.3	0	0	0	0	0.2
Nickel	110	99.7	0.3	0	0	0	0	0.2
Nickel	150	96.7	2.1	1.2	0	0	0	0.9

* Overall percentage of deuterium incorporated in molecule.

Samples at 50°C and 150°C were shaken mechanically.

Reagents: 10 mg catalyst, 0.055 moles D_2O, 0.002 moles chlorobenzene.

TABLE 2 DEUTERATION OF CHLOROBENZENE

CATALYST	TEMP. °C	Deuterium Incorporation (% Total)						% D *
		D_o	D_1	D_2	D_3	D_4	D_5	
Rhodium	50	99.9	0.1	0	0	0	0	0.1
Rhodium	110	96.4	2.6	0.1	1.0	0	0	1.2
Rhodium	150	92.9	2.5	2.7	1.9	0	0	2.7
Ruthenium	50	99.8	0.2	0	0	0	0	0.1
Ruthenium	110	99.6	0.4	0	0	0	0	0.2
Ruthenium	150	95.9	2.3	1.8	0	0	0	1.2
Iridium	50	94.8	4.6	0.1	0.6	0	0	1.2
Iridium	110	84.6	13.2	0.4	1.0	0.8	0	4.1
Iridium	150	54.8	28.7	11.9	4.6	0	0	13.2

* Overall percentage of deuterium incorporated in molecule.

Samples at 50°C and 150°C were shaken mechanically.

Reagents: 10 mg catalyst, 0.055 moles D_2O, 0.002 moles chlorobenzene.

Deuterium Exchange of Aromatic Halogen Derivatives

A. Chlorine and Bromine substituted Aromatic Compounds

On platinum the heterogeneous exchange (Table 1) of chlorobenzene exhibits a cut-off in the deuteration pattern at D_3, corresponding to the incorporation of three deuterium atoms in the molecule. This cut-off can be explained by ortho deactivation by chlorine[16]. Garnett interprets these ortho deactivation effects as being due to difficulties in forming a σbond at the carbon adjacent to chlorine on the metal surface (reaction 8) due to the physical bulk of the chlorine substituent. Hannan[32] has also found ortho deactivation of chlorobenzene with platinum catalysts of widely varying activity, prepared by different methods of catalyst activation.

Other metals of Group VIII (Palladium, Nickel, Rhodium, Ruthenium and Iridium) have also been examined as catalysts for the deuterium exchange of chlorobenzene (Tables 1 and 2). Of the five other metals examined only iridium produces an overall deuterium incorporation approaching that of platinum. The ortho deactivation effect is again apparent with iridium especially at the higher temperatures. Palladium and nickel do not exhibit appreciable activity but rhodium and ruthenium do exhibit some low level incorporation, especially at the higher temperatures. The overall level of incorporation is however too low to determine if ortho deactivation is occurring as a significant process. With both platinum and iridium the small amount of deuteration beyond D_3 indicates that chlorine deactivation is "severe" but not as complete as that reported for the position flanked by two methyl groups of m-xylene[15].

TABLE 3 DEUTERATION OF BROMOBENZENE

CATALYST	TEMP. °C	Deuterium Incorporation (% Total)						% D
		D_o	D_1	D_2	D_3	D_4	D_5	*
Platinum	50	96.2	3.5	0.1	0.2	0	0	0.8
Platinum	110	88.6	11.4	0	0	0	0	2.3
Platinum	150	81.4	18.3	0.3	0	0	0	3.8
Palladium	50	99.4	0.4	0	0	0	0	0.2
Palladium	110	98.6	1.4	0	0	0	0	0.3
Palladium	150	97.7	1.7	0.1	0.5	0	0	0.7
Nickel	50	97.3	2.4	0.1	0.2	0	0	0.6
Nickel	110	97.0	2.5	0.3	0.1	0	0	0.7
Nickel	150	94.8	4.7	0.1	0.4	0	0	1.2

* Overall percentage of deuterium incorporated in molecule.

Samples at 50°C and 150°C were shaken mechanically.

Reagents: 10 mg catalyst, 5.5×10^{-2} moles D_2O, 1.9×10^{-3} moles bromobenzene.

TABLE 4 DEUTERATION OF BROMOBENZENE

CATALYST	TEMP. °C	Deuterium Incorporation (% Total)						% D *
		D_o	D_1	D_2	D_3	D_4	D_5	
Rhodium	50	98.4	1.2	0.1	0.3	0	0	0.5
Rhodium	110	98.5	1.3	0.1	0.2	0	0	0.5
Rhodium	150	97.8	0.3	1.8	0.1	0	0	0.9
Ruthenium	50	99.8	0.2	0	0	0	0	0.1
Ruthenium	110	98.7	1.1	0.1	0.1	0	0	0.3
Ruthenium	150	96.7	2.1	1.2	0	0	0	0.9
Iridium	50	94.9	4.6	0.1	0.4	0	0	1.2
Iridium	110	89.8	9.3	0.1	0.8	0	0	2.4
Iridium	150	69.8	11.8	16.3	2.1	0	0	10.2

* Overall percentage of deuterium incorporated in molecule.
Samples at 50°C and 150°C were shaken mechanically.
Reagents: 10 mg catalyst, 5.5×10^{-2} moles D_2O, 1.9×10^{-3} moles bromobenzene.

Fraser and Renaud[13], Hannan[32] and Brown and Garnett[33] have observed that bromobenzene exchange on platinum is very slow in comparison to other organics such as chlorobenzene. These observations are consistent with the results obtained (Table 3), a general low level of deuterium incorporation for the exchange of bromobenzene with platinum as catalyst.

For other metals (Tables 3 and 4), with the exception of iridium, the reactivity is even less than that observed the platinum as catalyst. This slow exchange may be due to a combination of factors. Bromobenzene has a relatively low ionisation potential, thus the delocalization of π-electrons occurs more readily to the catalyst and the molecule is more strongly adsorbed. The observed slow exchange can thus be attributed to the displacement of the second reagent (heavy water as a source of deuterium) from the catalyst surface. A similar connection between ionisation potential and stability of the homogeneous complexes involving silver ions and halogens has been reported[34]. The reported[14,35] ability of the halogen substituents in halobenzenes to undergo charge transfer adsorption through the halogen atom (species V) may be occurring and supplementing the π-complex adsorption. Thus the molecule would be more strongly adsorbed on the catalyst surface displacing the second reagent (heavy water).

Iridium catalysed exchange of bromobenzene exhibits a higher level of incorporation of deuterium than that observed with the other metals (Table 4). This is similar to the effect observed with chlorobenzene on iridium (Table 2). This may be explained by the formation of a less stable π-complex between iridium and bromobenzene than that formed with the other metals examined, thus promoting exchange.

TABLE 5 DEUTERATION OF 2-CHLOROTOLUENE

CATALYST	TEMP. °C	Deuterium Incorporation (Percentage of Total)								% D *
		D_0	D_1	D_2	D_3	D_4	D_5	D_6	D_7	
Platinum	50	51.9	29.1	16.1	1.3	0.4	0	0	0	10.3
Platinum	150	2.2	9.0	65.7	17.5	4.6	1.0	0	0	30.9
Palladium	50	97.4	1.5	0.1	0.8	0.2	0	0	0	0.7
Palladium	150	88.9	8.8	0.1	1.3	0.9	0	0	0	2.3
Nickel	50	96.8	1.0	1.2	1.0	0	0	0	0	1.0
Nickel	150	91.1	1.0	5.9	2.0	0	0	0	0	2.7
Rhodium	50	97.8	1.4	0.1	0.7	0	0	0	0	0.5
Rhodium	150	96.1	3.2	0.1	0.6	0	0	0	0	0.7

* Overall Percentage deuterium incorporated in the molecule.

All samples were shaken mechanically.

Reagents: catalyst 10 mg, 5.5×10^{-2} moles D_2O, 1.7×10^{-3} moles 2-chlorotoluene.

Some exchange runs were also performed with ring substituted bromotoluenes on platinum. Results similar to those obtained with bromobenzene on platinum (Table 3) were observed, but the overall level of incorporation was correspondingly lower, expecially for 4-bromotoluene where ortho deactivation by both bromine and methyl would be expected.

For the exchange on platinum of 2-chlorotoluene a cut-off at the D_2 level in the deuteration pattern was observed (Table 5). PMR analysis shows (Table 6) that the deuterium incorporation into the

TABLE 6

Distribution of deuterium between ring and side chain for 2-chlorotoluene, platinum catalyst, 50°C. *

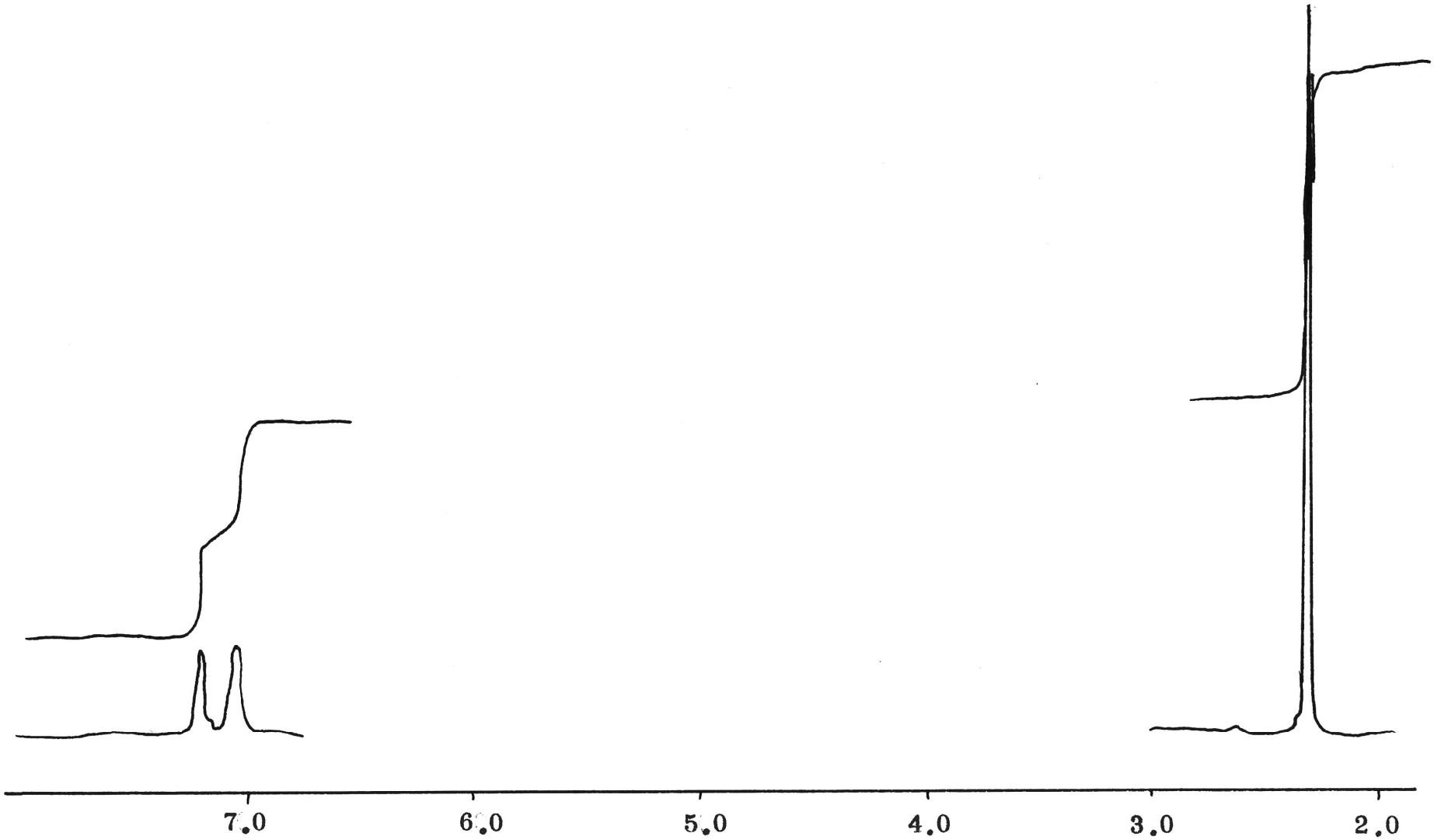
	Aromatic Ring	Methyl Group
Initial %H	57.1	42.9
%H after deut.	48.6	41.1
%D incorporated	8.5	1.8

* area ratios of pmr signals calculated by integration overall deuterium incorporation 10.3%.

molecule has been predominantly in the aromatic ring, when the reaction was allowed to proceed at 50°C. With a correspondingly higher level of deuterium exchange in the molecule at 150°C, the incorporation of deuterium has been almost exclusively in the aromatic ring (Table 7).

FIGURE 2

PMR Spectrum of 2 Chlorotoluene
after exchange on platinum at 150°C



Thus the cut-off at the D_2 level can be attributed to ortho deactivation

TABLE 7

Distribution of deuterium between ring and side chain for 2-chlorotoluene, platinum catalyst, 150°C *

	Aromatic Ring	Methyl Group
%H initially	57.1	42.9
%H after deut.	26.4	42.7
%D incorporated	30.7	0.2

* 30.9% overall deuterium incorporation.

by both chlorine and methyl groups, leaving only two positions readily available for exchange in the aromatic ring. The PMR spectrum (figure 2) provides confirmation of this pattern as the signals in the aromatic region have been reduced to a pair of singlets. The combination of ortho deactivation and the high level of deuterium incorporation (approximately 50% of the four aromatic protons have been replaced by deuterium) in the remaining free ring positions (4 and 5) results in the protons at positions 3 and 6 being unaffected by the spins of vicinal protons and thus appearing as singlets in the pmr spectrum (figure 2). Due to the differing chemical shifts of protons vicinal to methyl and chlorine the singlets are not superimposed but rather appear separate. The electric quadrupole moment of deuterium causes protons spin coupled to it to give slightly broadened lines[36], thus accounting for the broadness of the singlets. The ortho deactivation is not complete but only "severe" as evidenced by the relatively small amount of incorporation beyond D_2, although in the run performed at 50°C this may in part be due to exchange of the methyl protons.

TABLE 8 DEUTERATION OF 3-CHLOROTOLUENE

CATALYST	TEMP. °C	Deuterium Incorpoaation (Percentage of Total)								% D *
		D_o	D_1	D_2	D_3	D_4	D_5	D_6	D_7	
Platinum	50	71.4	28.4	0.2	0	0	0	0	0	4.8
Platinum	150	48.3	48.5	0.9	0.1	1.9	0	0	0	8.6
Palladium	50	99.3	0.7	0	0	0	0	0	0	0.1
Palladium	150	97.8	1.4	0.1	0.7	0	0	0	0	0.6
Nickel	50	95.7	1.1	2.2	1.0	0	0	0	0	1.0
Nickel	150	93.5	1.2	3.9	1.4	0	0	0	0	2.0
Rhodium	50	98.7	0.4	0.9	0	0	0	0	0	0.3
Rhodium	150	98.1	1.8	0.1	0	0	0	0	0	0.4

* Overall percentage deuterium incorporated in the molecule.

All samples were shaken mechanically.

Reagents: catalyst 10 mg, 5.5×10^{-2} moles D_2O, 1.7×10^{-3} moles 3-chlorotoluene.

The other metals used (Palladium, Nickel and Rhodium) do not show the same degree of activity as does platinum (Table 5). With palladium and rhodium the low level of incorporation of deuterium and fluctuations in the mass spectrometer make assessment of the deuteration pattern difficult. Nickel exhibits a cut-off at the D_3 level. Later work (Table 19) shows that with the fluorotoluene series nickel predominantly exchanges the methyl protons rather than the aromatic protons. This is consistent with the reported results of Macdonald and Shannon[29] and Garnett[14] that nickel normally favours aliphatic hydrogens in mixed aromatic-aliphatic systems such as the alkylbenzenes. Thus the cut-off at the D_3 level can be explained as exchange of the methyl protons.

For the exchange of 3-chlorotoluene on platinum (Table 8) a D_1 cut-off is observed. This can be explained in terms of ortho deactivations since only position 5 on the aromatic ring would be unaffected. Thus only one deuterium atom could be incorporated in the aromatic ring. This is confirmed by the pmr spectrum (Table 9) which shows that the

TABLE 9

Distribution of deuterium between ring and side chain for 3-chlorotoluene, platinum catalyst, 150°C. *

	Aromatic Ring	Methyl Group
%H initially	57.1	42.9
%H after Deut.	49.1	42.3
%D incorporated	8.0	0.6

* overall deuterium incorporation 8.6%

deuterium incorporation is predominantly in the aromatic ring. Palladium

TABLE 10 DEUTERATION OF 2-FLUOROTOLUENE

CATALYST	TEMP. °C	Deuterium Incorporation (Percentage of Total)								% D *
		D_0	D_1	D_2	D_3	D_4	D_5	D_6	D_7	
Platinum	50	74.2	18.2	5.5	0.9	0.6	0.5	0.1	0	5.3
Platinum	150	3.3	13.7	42.1	36.6	3.6	0.2	0.2	0.3	32.4
Palladium	50	93.0	5.5	0.8	0.5	0.2	0	0	0	1.4
Palladium	150	2.0	6.9	16.7	25.9	26.6	16.9	4.7	0.4	48.5
Nickel	50	97.5	0.4	1.2	0.9	0	0	0	0	1.0
Nickel	150	59.1	18.8	11.7	9.4	0.8	0.2	0	0	10.6
Rhodium	50	95.7	2.6	0.9	0.3	0.3	0.2	0	0	1.0
Rhodium	150	63.1	22.5	7.2	5.3	1.5	0.3	0.1	0	8.7

* Overall percentage of deuterium incorporated in molecule. All samples were mechanically shaken.

Reagents: 10 mg catalyst, 5.5×10^{-2} moles D_2O, 1.8×10^{-3} moles 2-Fluorotoluene.

and rhodium appear to exhibit a D_1 cut-off but this is inconclusive due to the low level of incorporation with these catalysts (Table 8). Nickel again exhibits a cut-off at the D_3 level which can be attributed to the exchange of the three methyl protons as discussed above for 2-chlorotoluene.

The results obtained with chloro and bromobenzene and 2- and 3-chlorotoluene are consistent with the theory of ortho deactivation by methyl, chlorine and bromine.

B. Fluorine substituted aromatic compounds

The pattern of deuterium exchange amongst the fluorinated compounds studied is different from that observed with 2-and 3-chlorotoluene. For the deuteration of 2-fluorotoluene (Table 10) on platinum at cut-off at the D_3 level is evident whereas the corresponding results for 2-chlorotoluene (Table 5) show the cut-off at D_2. Thus the exchange of 2-fluorotoluene on platinum must proceed by a somewhat different mechanism that permits deuterium exchange of up to three positions readily and where ortho-deactivation does not play the same role as for 2-chlorotoluene. That these three positions are on the aromatic ring and not the methyl group is shown by the pmr data (Table 11). The exchange of

TABLE 11

Distribution of deuterium between ring and side chain for 2-fluorotoluene, platinum catalyst, 150°C. *

	Aromatic Ring	Methyl Group
%H initially	57.1	42.9
%H after deut.	28.3	39.3
%D incorporated	28.9	3.5

* overall deuterium incorporation was 32.4%

2-fluorotoluene on nickel exhibits a D_3 cut-off (Table 10). This is substantiated by the pmr measurements on 4-fluorotoluene (Table 19) and is consistent with the observations of other workers[14,29].

For the exchange of 2-fluorotoluene on palladium (Table 10) a different pattern again is observed. At 50°C there is only 1.4% overall deuterium incorporation but this rises to 48.5% and the deuteration pattern is spread up to D_6. This indicates that palladium incorporates some character of both platinum and nickel and thus deuteration would occur in both the aromatic ring and the methyl group. The pmr results (Table 12) indicate nearly equal incorporation of deuterium in both the

TABLE 12

Distribution of deuterium between ring and side chain for 2-fluorotoluene, palladium catalyst, 150°C. *

	Aromatic Ring	Methyl Group
%H initially	57.1	42.9
%H after deut.	32.2	19.3
%D incorporation	24.9	23.6

* overall deuterium incorporation 48.5%

aromatic ring and the methyl group. These results are consistent with the reported results for exchange on palladium in mixed aromatic-aliphatic systems[29,37]. The exchange of the methyl protons on palladium has been reported[24,38] as being due to the formation of π-allylic intermediates which promote exchange of these protons. Rhodium appears to exhibit a D_3 cut-off but the situation is not as clear due to the lower level of deuterium incorporation.

TABLE 13 DEUTERATION OF 3-FLUOROTOLUENE

CATALYST	TEMP. °C	Deuterium Incorporation (Percentage of Total)								% D *
		D_0	D_1	D_2	D_3	D_4	D_5	D_6	D_7	
Platinum	50	90.9	0.6	6.1	0.2	1.9	0.3	0	0	3.2
Platinum	150	14.9	42.0	39.9	2.8	0.3	0.1	0	0	18.9
Palladium	50	95.6	1.3	1.6	1.4	0.1	0	0	0	1.3
Palladium	150	63.9	19.3	2.7	7.2	0.7	0.2	0	0	8.8
Nickel	50	99.5	0.2	0.3	0	0	0	0	0	0.1
Nickel	150	78.4	10.9	6.3	4.2	0.2	0	0	0	5.3
Rhodium	50	97.2	2.8	0	0	0	0	0	0	0.4
Rhodium	150	92.9	5.6	0.8	0.4	0.1	0	0	0	1.4

* Overall percentage of deuterium incorporated in molecule.

All samples were shaken mechanically.

Reagents: 10 mg catalyst, 5.5×10^{-2} moles D_2O, 1.8×10^{-3} moles 3-Fluorotoluene.

The deuterium exchange pattern of 3-fluorotoluene on platinum catalyst (Table 13) shows a cut-off occurring at the D_2 level corresponding to the incorporation of two molecules of deuterium in the molecule. At 150°C on platinum the deuterium incorporation (Table 14) is predominantly in the aromatic ring, indicating that the cut-off at the D_2

TABLE 14

Distribution of deuterium between ring and side chain for 3-fluorotoluene, platinum catalyst, 150°C *

	Aromatic Ring	Side Chain
%H initially	57.1	42.9
%H after deut.	40.4	40.7
%D incorporated	16.7	2.2

* overall deuterium incorporation 18.9%.

level is due to incorporation of two deuterium atoms in the aromatic ring. Nickel again exhibits a D_3 cut-off due[14,29] to exchange of the methyl protons rather than the aromatic protons. The low level of incorporation for rhodium catalysed exchange precludes significant comment on the deuteration pattern.

With 3-fluorotoluene on palladium a two day run (Table 13) indicates a cut-off at the D_3 level which can be explained by a greater level of incorporation of deuterium in the methyl group than the aromatic ring. The pmr data (Table 15) is consistent with this explanation of the deuteration pattern.

TABLE 16 DEUTERATION OF 4-FLUOROTOLUENE

CATALYST	TEMP. °C	Deuterium Incorporation (Percentage of Total)								% D *
		D_o	D_1	D_2	D_3	D_4	D_5	D_6	D_7	
Platinum	50	96.2	1.5	1.1	0.5	0.4	0.3	0	0	1.2
Platinum	150	53.1	35.1	9.2	1.6	0.4	0.3	0.2	0.2	9.1
Palladium	50	95.7	1.9	2.3	0.1	0	0	0	0	1.0
Palladium	150	4.4	13.1	21.9	30.5	22.0	7.4	0.6	0	39.6
Nickel	50	69.9	18.8	6.9	4.3	0.1	0	0	0	6.5
Nickel	150	67.3	18.8	8.8	5.1	0	0	0	0	7.4
Rhodium	50	92.1	4.0	3.9	0	0	0	0	0	1.7
Rhodium	150	90.6	7.0	1.3	1.0	0.1	0	0	0	1.9

* Overall percentage of deuterium incorporated in molecule.

All samples were mechanically shaken.

Reagents: 10 mg catalyst, 5.5×10^{-2} moles D_2O, 1.8×10^{-3} moles 4-Fluorotoluene.

TABLE 15

Distribution of deuterium between ring and side chain for 3-fluorotoluene, palladium catalyst, 150°C.*

	Aromatic Ring	Methyl Group
%H initially	57.1	42.9
%H after deut.	56.2	35.0
%D incorporated	0.9	7.9

* overall deuterium incorporation 8.8%. Sample kept at 150°C for two days.

The exchange of 4-fluorotoluene on platinum (Table 16) shows a cut-off in the deuteration pattern at the D_2 level. The pmr data shows that the incorporation of deuterium in the molecule has been mainly in the

TABLE 17

Distribution of deuterium between ring and side chain for 4-fluorotoluene, platinum catalyst, 150°C.*

	Aromatic Ring	Methyl Group
%H initially	57.1	42.9
%H after deut.	51.4	39.5
%D incorporated	5.7	3.4

* overall deuterium incorporation 9.1%.

aromatic ring (Table 17). However the situation is not as clear as before with some exchange of the methyl protons occurring. Some other factors not in evidence previously may thus be significant in this case. Rhodium appears to also exhibit a cut-off at the D_2 level but the low level of incorporation (Table 16) again precludes significant comment.

Palladium again shows a low level of incorporation at 50°C and significant incorporation up to D_5 at 150°C. Most of the deuterium incorporation has been in the methyl group (Table 18) for the exchange of 4-fluorotoluene at 150°C on palladium. However 23% of the total deuterium incorporation is present in the aromatic ring. These results

TABLE 18

Distribution of deuterium between ring and side chain for 4-fluorotoluene, palladium catalyst, 150°C. *

	Aromatic Ring	Methyl Group
%H initially	57.1	42.9
%H after deut.	48.0	12.4
%D incorporated	9.1	30.5

* overall deuterium incorporation 39.6%.

can be explained again in terms of the π-allylic bonding character of palladium as discussed for 2-fluorotoluene.

The deuteration pattern of 4-fluorotoluene on nickel shows a cut-off at the D_3 level (Table 16). The pmr data for the exchange at 150°C (Table 19) shows that the incorporation is almost exclusively in the

TABLE 19

Distribution of deuterium between ring and side chain for 4-fluorotoluene, nickel catalyst, 150°C.

	Aromatic Ring	Methyl Group
%H initially	57.1	42.9
%H after deut.	57.0	35.6
%D incorporated	0.1	7.3

overall deuterium incorporation 7.4%.

methyl group. This is consistent with the reported work of Macdonald and Shannon[29], Garnett[14] and Hirota and Veda[39] that nickel selectively deuterates the side chain protons in mixed aromatic-aliphatic systems.

Thus the results for fluorotoluenes do not conform to the pattern established for halobenzenes by Garnett and Sollich[16] and Brown and Garnett[33]. Fraser and Renaud[13] on the basis of their protium exchange of highly deuterated aromatics propose only slight steric effects due to fluorine but severe ortho deactivation due to chlorine and bromine. They explain this process by comparing the carbon-fluorine bond length in fluorobenzene (1.33A^{o}) to the carbon hydrogen bond length of 1.08Å in benzene. They also compare the van der Waals radii for fluorine (1.35Å) and hydrogen (1.2Å) and draw the conclusion that fluorine is only slightly larger than hydrogen and thus its steric effect ought to be small. Garnett and Sollich[16] observe ortho deactivation of all halobenzenes but latter work by Garnett[14] suggests that fluorine may be an exception. Garnett and Sollich - Baumgartner[31,40] show ortho deactivation adjacent to methyl groups on aromatic rings (such as toluene, xylenes).

Thus positions ortho to a methyl group are deactivated but positions ortho to fluorine on an aromatic ring are at worst slightly deactivated. The deuteration patterns observed for the fluorotoluenes on platinum can thus be explained. For 2-fluorotoluene there are four ring positions remaining not substituted by methyl and fluorine. The proton at position 6 will be deactivated towards exchange by the methyl group, and thus the incorporation of three aromatic protons would be expected. The experimental results (Table 10) are consistent with this pattern. For 3-fluoro-

toluene, positions 2 and 6 will be deactivated by reason of the methyl ortho deactivation, and two positions are then left free for exchange. Experimental verification is provided for these patterns with platinum catalyst (Table 13). Similarly for 4-fluorotoluene positions 2 and 6 are ortho deactivated by methyl and positions 3 and 5 are free for exchange but ortho to fluorine. Under the same conditions of time and temperature (1 week and 150° respectively) the overall percentage of deuterium incorporation decreases from 2-fluorotoluene to 4-fluorotoluene (Table 20). This can be attributed to the decrease in unhindered ring

TABLE 20

Relative rates of deuteration of fluorotoluenes at 150°C on platinum catalyst.

COMPOUND	OVERALL %D
2-fluorotoluene	32.4
3-fluorotoluene	18.9
4-fluorotoluene	9.1

protons, from two to zero respectively. Unhindered ring protons are those protons flanked on both sides by other ring protons. Thus 2-fluorotoluene contains two unhindered protons, 3-fluorotoluene one, and 4-fluorotoluene none. Although the small size of the fluorine atom would indicate no steric hindrance[13], there is obviously a factor which causes a lowering of the percentage deuteration ortho to fluorine. This effect is however not nearly as severe as that exhibited by chlorine or bromine.

The results for some deuterium exchange reactions with fluorobenzene and Group VIII metal catalysts confirm these results (Tables 21 and 22). Platinum, Palladium and Iridium are active as catalysts for deuterium

TABLE 21 DEUTERATION OF FLUOROBENZENE

CATALYST	TEMP. °C	Deuterium Incorporation (% Total)						% D *
		D_o	D_1	D_2	D_3	D_4	D_5	
Platinum	50	95.0	3.8	0.9	0.2	0	0	1.3
Platinum	110	23.0	21.9	19.1	18.5	12.4	5.2	38.2
Platinum	150	3.2	17.4	31.6	29.9	14.9	3.1	49.0
Palladium	50	76.4	19.7	3.0	0.7	0.2	0	5.7
Palladium	110	13.0	33.9	30.9	15.5	5.8	1.1	34.1
Palladium	150	1.2	7.4	19.1	33.2	31.1	8.0	61.9
Nickel	50	99.6	0	0.3	0	0	0	0.2
Nickel	110	99.2	0.4	0.5	0	0	0	0.3
Nickel	150	99.2	0.3	0.3	0.2	0	0	0.3

* Overall percentage of deuterium incorporated in molecule.

Samples at 50°C and 150°C were shaken mechanically.

Reagents: 10 mg catalyst, D_2O 5.5×10^{-2} moles, Fluorobenzene 2.7×10^{-3} moles.

TABLE 22 DEUTERATION OF FLUOROBENZENE

CATALYST	TEMP. °C	Deuterium Incorporation (% Total)						% D *
		D_o	D_1	D_2	D_3	D_4	D_5	
Rhodium	50	99.4	0.3	0.3	0	0	0	0.2
Rhodium	110	98.0	1.5	0.5	0	0	0	0.6
Rhodium	150	76.3	19.4	3.0	0.8	0.5	0	5.9
Ruthenium	50	99.1	0.5	0.4	0	0	0	0.3
Ruthenium	110	99.0	0.4	0.4	0	0.1	0	0.4
Ruthenium	150	95.4	3.5	0.8	0.3	0	0	1.2
Iridium	50	99.3	0.5	0.3	0	0	0	0.2
Iridium	110	85.2	10.1	2.4	0.9	0.8	0.6	4.7
Iridium	150	42.2	31.7	15.0	6.8	3.2	1.1	20.1

* Overall percentage of deuterium incorporated in molecule.

Samples at 50°C and 150°C were shaken mechanically.

Reagents: 10 mg catalyst, D_2O 5.5×10^{-2} moles, Fluorobenzene 2.7×10^{-3} moles.

exchange of fluorobenzene. Rhodium and Ruthenium exhibit a small degree of exchange whilst nickel is practically inactive as a catalyst. With the more active catalysts the deuteration pattern at the lower temperatures especially indicates that there is some barrier to the incorporation of a fourth and fifth deuterium atom in the ring. This suggests that the positions ortho to fluorine are not completely equivalent to the other protons on the benzene ring. However with the higher temperature (150°C) deuteration of fluorobenzene with platinum and palladium this phenomenon disappears indicating that the restriction to exchange in these positions is slight.

Chlorobenzene and bromobenzene show little deuterium exchange activity on Palladium (Tables 1 and 3). However with fluorobenzene palladium is very active producing a higher degree of deuterium incorporation than that observed with platinum. This may be explained by the formation of a more stable π-complex between palladium and chloro and bromobenzenes than that with fluorobenzene. The more stable the π-complex the greater the tendency for the molecule to remain complexed on the surface and thus limit exchange by displacement of the source of deuterium (heavy water). The reactivity of the fluorotoluenes with palladium can further be explained by the tendency[24,38] of palladium to form π-allylic complexes (species VI) which will promote exchange of the methyl protons.

The results obtained with palladium catalysed exchange of fluorobenzene show a marked temperature effect (Table 21). A temperature of 110°C or 150°C will produce substantial incorporation of deuterium into the fluorobenzene molecule. This effect occurs with all exchange re-

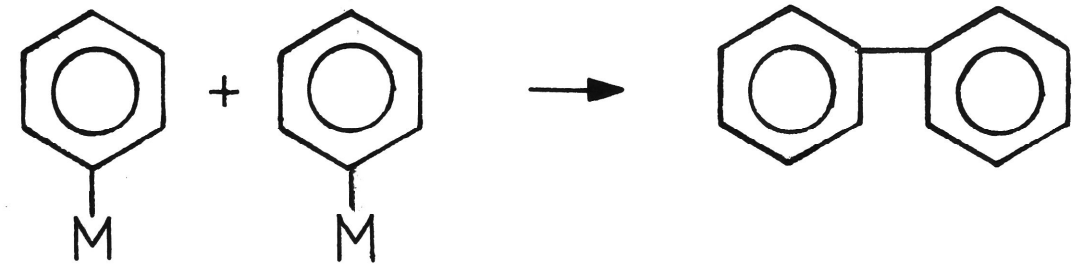
actions studied but is more obvious with those catalysts that produce a significant amount of exchange (Platinum, Palladium, Iridium and nickel for toluene derivatives). At the higher temperatures the higher level of deuterium incorporation may be accompanied by appreciable by-product formation.

C. Side Reactions and By-products

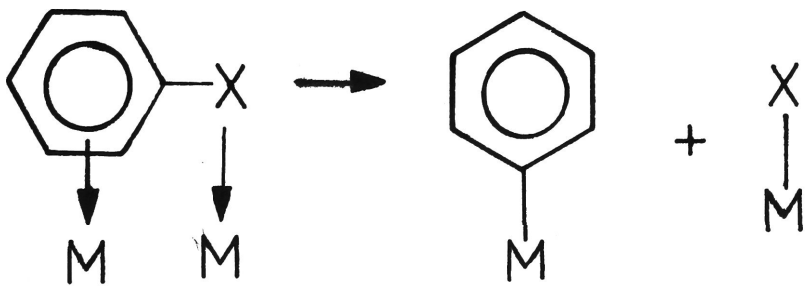
Side reactions have been associated with the catalytic exchange of organic compounds with deuterium gas[16]. Competing hydrogenation of the organic substance being examined may yield high proportions of by-products especially on evaporated metal films[9]. Whilst these products complicate the identification of deuterated species, when exchange using tritium is undertaken the highly active products formed render extremely difficult or even preclude radiochemical purification of the parent compound[41-43]. A change in reaction conditions to the use of heavy water as a source of deuterium has been favoured in order to reduce the complications from the formation of by-products[9,16].

That some side reactions do occur during exchange with heavy water has been shown by Garnett and co-workers[8,14,32,44]. The presence of some by-products accompanying deuteration of fluorobenzenes was shown by semi-quantitative gas chromatography. GC-MS of the organic layer of the reaction mixture showed that these by-products were predominantly mono - or di-fluorobiphenyl with traces of higher molecular weight compounds. Garnett and Calf[44] have shown biphenyl formation during heterogeneous exchange on platinum of benzene. They explain this as being due to a competing reaction whereby two sigma bonded benzene molecules desorb from the metal surface with the formation of a carbon-carbon bond to give a

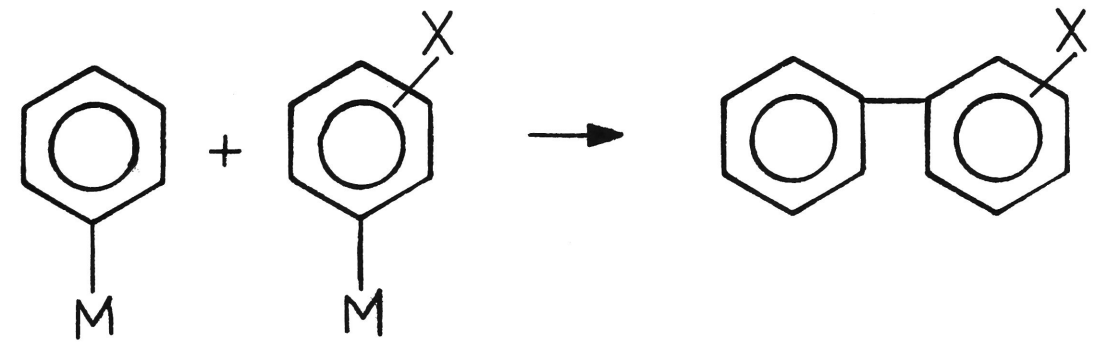
FIGURE 3



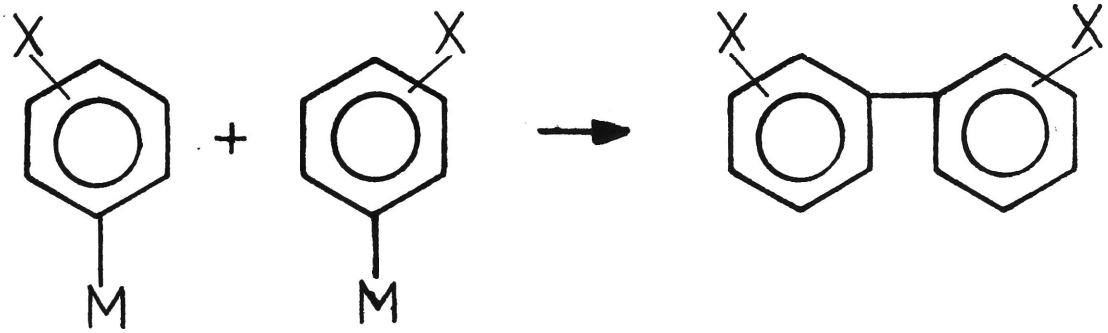
(10)



(11)



(12)



(13)

molecule of biphenyl (reaction 10). Fluorinated benzenes may undergo a similar reaction (reaction 13) or alternatively if the carbon-fluorine bond breaks (reaction 11) the formation of phenyl radicals on the catalyst surface would occur and combination of these with sigma bonded fluorobenzene (reaction 12, X = F) would explain the formation of monofluorodiphenyls.

With chlorobenzene and bromobenzene dehalogenation appears to be the predominant side reaction which may be explained in terms of carbon-halogen bond breakage (reaction 11, X = Cl, Br) but no polymerisation by combination reactions. With the chlorotoluenes the use of GCMS detected small amounts of by-products of mass 140 and 160. These can be attributed to chloroxylenes and dichlorotoluenes formed by the demethylation and dechlorination of chlorotoluene and the combination of these groups with chlorotoluene molecules on the catalyst surface similarly to that observed with flurobenzene (reactions 11, 12). Traces were also found on occasions of toluene, chlorobenzene, xylene and chlorotrimethylbenzene.

The overall levels of by-product were generally low but with nickel catalyst the level of dehalogenation was quite high. The nickel catalyst, presumably undergoing attack changed character from the usual grey black precipitate to a bluish-green coloured sludge, with attendent loss of activity.

Methods of Deuterium Analysis

A number of methods have been used to analyse for total percentage deuterium and positional distribution of deuterium incorporation in organic molecules. The methods all depend on a significant difference between the physical properties of hydrogen and deuterium in order to eliminate interference from normal hydrogen (protium).

A. Combustion Method

Two combustion methods have, in the past, been used[1,45], one involving combustion of the sample to molecular hydrogen (deuterium) gas and the other to water. The compounds are then analysed for their deuterium content, by detecting a change in a physical quantity which is a function of the deuterium content. For analysing the hydrogen gas a mass spectrometer is often used [45,46]. The latter method[45] of combustion to water and then reduction is the more accurate of the two due to lesser "memory" effects, higher precision (0.001%) and smaller sample size necessary.

Combustion methods, although having high precision suffer from the disadvantage of requiring at least several milligrams of sample. This sample must be extremely pure to take advantage of the high precision and the possibility of erroneous results from contamination or incomplete combustion is always present. Positional identity and abundance are lost by combustion of the organic sample.

It would seem that combustion of a sample containing deuterium to water and measurement of the isotopic abundance of this water in a mass spectrometer would be a relatively simple analysis technique. However Gaff and Ritenberg[45] have shown that if pure deuterium oxide is introduced

into a baked-out mass spectrometer a spectrum somewhat different to that expected will occur (Table 23). The sample of deuterium oxide has become

TABLE 23

Typical relative intensities of mass 16-20 before and after introduction of deuterium oxide

Mass	Background	after D_2O
16	0.3	94
17	0.8	43
18	2.8	702
19	0	777
20	0	1000

diluted with normal hydrogen atoms from compounds present in the mass spectrometer. These hydrogen atoms are present as either adsorbed water or hydroxyl groups of the silicates of the glass vacuum chamber. Thus the analysis of water directly in a mass spectrometer is not feasible.

B. INFRA-RED ANALYSIS

Another method of estimation of the deuterium content of an organic compound is by comparison of the position and intensity of bands in the IR spectrum of the deuterated substance with those of the undeuterated substance. Whiffen and Thompson[47] have shown that there is a correlation between IR spectra and molecular structure they also examined the effect of substituent groups on the basic benzonoid frequencies. Thompson and co-workers[48,49] have examined the intense absorptions in the 700 - 900 cm^{-1} region for substituted benzenes and have found these bands characteristic of the position of the substituent and almost independent of their identity.

These authors have assigned the bands as carbon-hydrogen out-of-plane bending vibrations. Tiers and Tiers[50] have applied these results to the analysis of deuterated benzenes and substituted benzenes. They report that the deuterated benzenes have IR absorbances approximately 20 cm^{-1} above that of the corresponding undeuterated compounds. Thus the bands reported by Whiffen and Thompson[47] at 750-780, 790-810 and 820-850 cm^{-1} for ortho, meta and para substituted benzenes respectively can be used for measurement of the deuterium content of a substituted benzene.

The bands due to C-H and C-D stretching frequencies in the 220-3100 cm^{-1} region have also been used[16] but they are less intense and a correspondingly higher deuterium content is necessary before they can be utilised.

The main problem that lead to the disuse of IR analysis for deuterium is the closeness of the adjoining regions of absorption due to ortho, meta and para hydrogens in a benzene ring. These bands are within the shift (20 cm^{-1}) caused by deuterium substitution which can lead to difficulty in assignment of individual bands.

C. Proton Magnetic Resonance Analysis

Fraser and Renaud[13] have shown that PMR techniques can be used to examine the protium exchange of highly deuterated compounds. Here the proton signals are much reduced in intensity and the technique of "massive deuteration"[36,51] is used to advantage. In this way at a level of 75-95% deuterium incorporation the pmr absorption is due to protons whose vicinal substituents are mainly deuterium atoms. The pmr spectrum then shows only the much finer splittings characteristic of coupling to deuterium, which if necessary can be wholly eliminated by H-D spin decoupling[13,36]. The pmr spectrum then enables the proton distribution to be evaluated and by

subtraction the atom percentage protons introduced into each position can be found.

It is well known that pmr is useful for determination of the positional distribution of protons of aromatic compounds. From the areas obtained by integration of the methyl and aromatic signals in the pmr spectrum of a deuterated halotoluene a measure of the relative amounts of residual protium in these groups was obtained. The corresponding absolute amounts of residual protium were obtained from these by making use of the mass spectrometrically determined average deuterium (and hence average protium) content of the molecule as a whole. The distribution of deuterium incorporation in the molecule can then be obtained by subtraction of residual protium from that present in the undeuterated molecule. This method is similar to that reported by Macdonald and Shannon[29].

The determination of the distribution of deuterium around a monosubstituted benzene ring for deuterated compounds is however not as simple as determination of ring to side chain distribution. This is due to the presence of an envelope of aromatic protons whose pmr signals cannot be resolved in many cases. This envelope may render difficult or even preclude signal area determination by integration to establish deuterium distribution. However the literature[52,53] indicates that use of shift reagents should separate the respective signals sufficiently to enable their integration.

Shift reagents are used in pmr spectroscopy to reduce the equivalence of nuclei by altering their magnetic environment and are of two types: aromatic solvents such as benzene and paramagnetic metal complexes. The latter are of interest to the problem of determination of ortho, meta and

para proton ratios in substituted toluenes. Lanthanide shift reagents function by co-ordinating to suitable donor atoms in the molecule under study, thereby expanding their coordination shell and forming a new complex in solution. Apart from effects due to shielding by bonding electrons, the chemical shifts are altered by the so called pseudo-contact shift[53], due to the magnetic effects of the unpaired electron magnetic moment of the paramagnetic metal ion. Hinckley[52] in 1969 published the first article on lanthanide-metal complexes as shift reagents and since then a large number of articles have appeared including a review by Mayo[53]. Fluorinated acetylacetone complexes of lanthanides have been found[53] to give the greatest spectral shifts with an acceptable degree of line broadening.

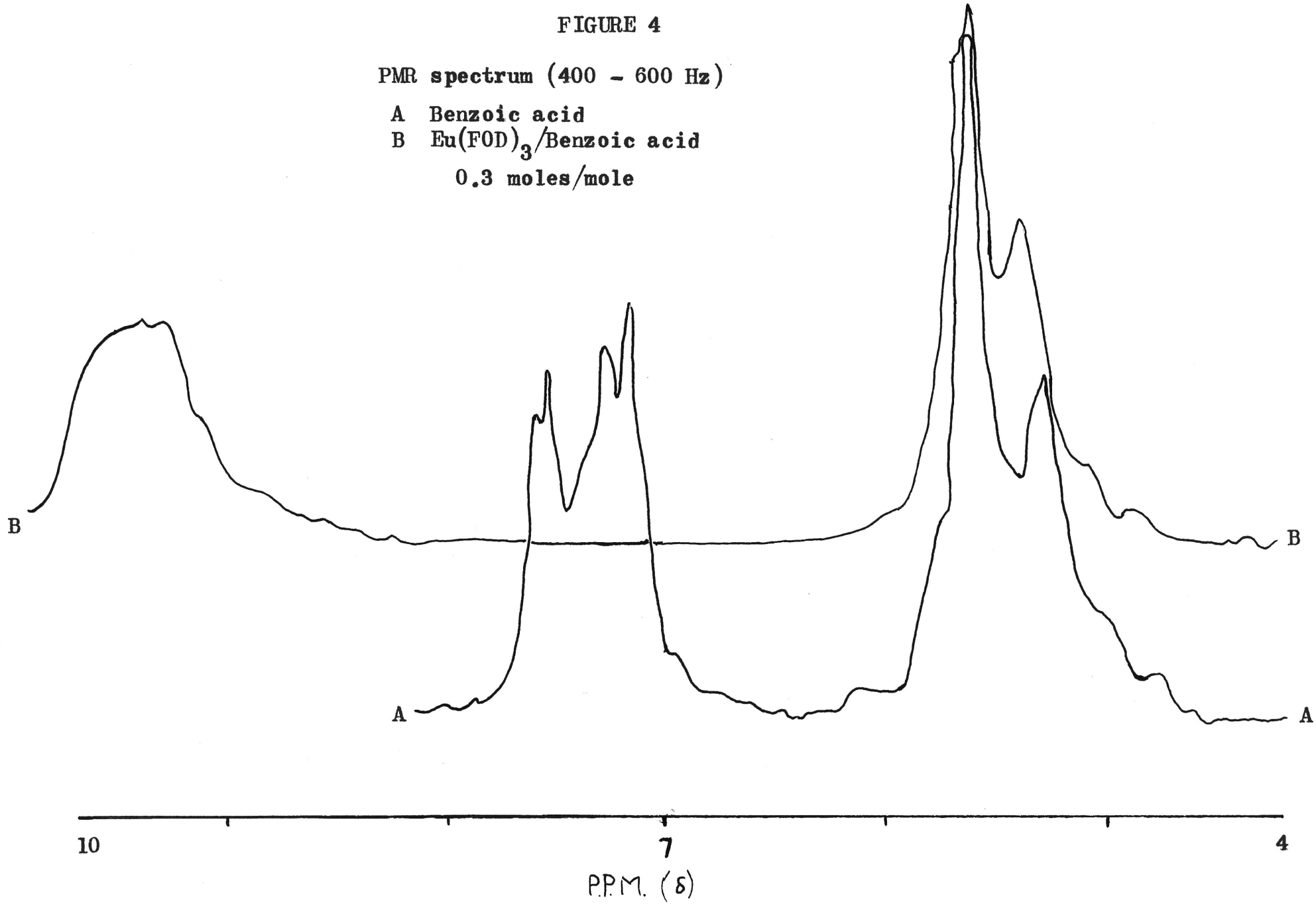
To make use of lanthanide shift reagents (LSR) for the determination of proton distribution by spectral simplification a suitable Lewis base for the lanthanide metal to coordinate to must be present. For alkyl benzene derivaties the side chain can be oxidised to a carborylic acid group which provides a suitable lewis base for coordination (oxygen). The literature suggests[54,55] that an increase in chemical shift occurs when protium is replaced by deuterium possibly due to an increase in base strength. Alkyl halides have been reported[56] to coordinate weakly or not at all. LSR have been used to simplify the spectra of aromatic compounds[56,57], notably separating the aromatic protons of quinoline[56]. Tris (6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionata) europium (III), commonly abbreviated as $Eu(FOD)_3$, is recommended by Shoeffner[58] as a shift reagent for use with carboxylic acids and phenols.

Thus $Eu(FOD)_3$ should be useful for simplification of the spectra of

FIGURE 4

PMR spectrum (400 - 600 Hz)

A Benzoic acid
B $Eu(FOD)_3$/Benzoic acid
0.3 moles/mole



deuterated halogenated benzoic acids. Since the compounds of interest were to be oxidised to benzoic acid derivatives by the method of Vogel[59], pure benzoic acid was selected as a trial compound to evaluate the method. From the work of Sanders and Williams[56] a lanthanide shift reagent to benzoic acid molar ratio of 0.3 or less should separate the ortho, meta and para proton signals of benzoic acid sufficiently to enable separate integration of their signals. The maximum shift obtained (Table 24) for

TABLE 24

Chemical shift changes upon addition of $Eu(FOD)_3$ to benzoic acid in carbon tetrachloride.

Ratio of lanthanide shift reagent to benzoic acid (moles/moles)	Chemical shift Hz
0.03	5
0.09	16
0.19	32
0.30	50

the ortho protons of benzoic acid was 50 Hz. The meta and para protons were not sufficiently well separated (figure 4) to enable accurate determination of their areas by integration. A normal pmr spectrum of benzoic acid can be integrated to obtain the ortho to meta/para proton ratio and thus the results obtained show no significant improvement from an integration of the non LSR spectrum. Time precluded further exploration of the problem, the solution may have been found in the use of other Europium complexes[53], change in the lanthanide metal such as praesodymium or change from the fluorinated acetylacetone type of shift reagent to some other type of shift reagent[54].

D. Mass Spectrometric Analysis

Although isotope ratio mass spectrometers[5,45] are used in the combustion method to analyse for deuterium a more general method for the determination of abundance and position of enrichment of stable isotopes in an organic molecule is that of direct analysis[1,3-6]. This technique involves measurement of ion intensities of the several isotopic species of a molecular or fragment ion formed in a mass spectrometer. In addition if structural information is required a study of fragmentation reactions through the use of normal and specifically labelled compounds may also be necessary. Deuterium analyses are conveniently performed by this method due to the increase of one atomic mass unit for every deuterium atom incorporated in the molecule.

The measurement of the specific ions of interest can be accompanied by alternately focussing these ions onto the ion detector of the mass spectrometer whilst the sample is supplied to the ion source. This alternating focusing must be performed at a rate sufficient to obtain many measurements at the ion signals whilst the sample is in the machine. This procedure results in an increased precision in abundance values and is accomplished by accelerating voltage adjustment in a magnetic sector instrument or by DC/RF voltage ratio adjustment in a quadrupole mass spectrometer.

Direct analysis was the method adopted for deuterium enrichment determination of the organic compounds studied in this thesis. With mass spectrometric analysis for deuterium care must be taken to keep the formation of fragment ions (especially M-1) to a minimum so as to reduce the contribution from ions of higher mass to the intensity of a particular

ion. This is generally achieved by operating at an ionizing voltage (electron energy) barely above the appearance potential of the ions of interest. The electron energy is then generally about 10-12 eV (electron volts). However at this point in the ionization efficiency versus electron energy curve the ionization efficiency changes quite markedly with small changes in the electron energy. This has been attributed[64] to the relatively large ($\frac{1}{4}$-$\frac{1}{2}$ eV) maxwellian thermal energy spread of the electrons emitted from the ionization source (filament). This variation in electron energy will tend to produce some fluctuations in the ion current due to fluctuations in the ionization efficiency of the process.

The Quadnova operating system, an "active" on-line computerised quadrupole mass spectrometer, was used for direct analysis for deuterium content of the labelled compounds prepared.

ISOTOPE RATIO DETERMINATIONS WITH A COMPUTER CONTROLLED QUADRUPOLE MASS SPECTROMETER

Introduction

Quadrupole mass analysers present some attractive features in the pursuit of higher precision isotope ratio measurements. Firstly the electrostatic voltages used to produce mass dispersion can be switched rapidly and measured accurately. The high switching rates mean that for each mass value the ion current can be collected for a time of the order of milliseconds. Since the dead or setting time between masses is less than one millisecond almost all the time the sample is in the instrument can be spent collecting data. Secondly, ions in a particular mass series may, if necessary, be collected for differing amounts of time depending on their relative abundances. Thirdly any ions in the spectrum, no matter what their mass difference, can be measured in a single analysis and need not lie within a given percentage of the mass range as is generally necessary for magnetic sector instruments.

The Quadnova System

An "active" on-line computerised mass spectrometer system (Quadnova operating system) has been developed at Wollongong University College to operate a gas chromatograph-quadrupole mass spectrometer-digital computer system in an interactive mode. The manual method of operation of the quadrupole mass spectrometer involves the taking of an oscillographic trace of the mass spectrum, developing the trace and analysing the spectrum by manual peak counting. In the computer mode a series of spectra over a given mass range are automatically recorded and stored on

magnetic tape. The spectra are then accessible for data analysis and interpretation without the need for peak counting. This system greatly simplifies the recording of large amounts of data from mass spectra of organic compounds, especially during G.C. runs.

The Quadnova system consists of five operational segments, CALIBRATE, SCAN, FETCH, BACKGROUND and DISPLAY together with a master program CONTROL whose function is to bring into operation the user's choice of segment. There are two large buffers within the system, the A-buffer (active) and the B-buffer (background), each of which can contain the intensity values for a complete mass spectrum of up to 650 mass values. The A-buffer is used to store the mass spectrum intensity data as it is collected in the SCAN segment and the B-buffer is used to store a background spectrum for subtraction during DISPLAY. The whole of the Quadnova software fits into memory (8K) at the same time.

The CALIBRATE segment is used to calibrate the computer software against the mass spectrometer. The use of a reference gas (perfluorotributylamine, FC43) enables the position of mass peaks to be determined as voltages set at the D/A interface.

The SCAN segment is the primary data collection routine. It allows the user to set various operating parameters on the mass spectrometer, causes the M.S. to scan over a given mass range for a given number of times, writes the collected data onto magnetic tape and draws an on-line reconstructed G.C. plot. Mass values (M-values) are set on the M.S. as voltages. The software converts a mass value to an integral number (termed an N-value) proportional to the voltage set by the D/A converter. N-values corresponding to each integer mass value in the required mass

range are passed in turn to the D/A converter during a SCAN operation. During the CALIBRATE routine the centroid in the N-value dimension of each reference gas mass peak is determined as an indicator of the centre of the peak. N-values are stored from CALIBRATE for each reference gas mass peak. In SCAN when an intensity has to be collected at a mass value the program converts the M-value to an N-value by the use of these reference gas peak N-values. The M-value is checked to see between which pair of reference gas peaks it lies and a linear interporlation relationship for M to N conversion is then used. By this means the effect of any small deviation from linearity of the instrument system will be reduced.

The FETCH routine collects a data block containing one mass spectrum plus 50_{10} words of identifying information and writes it into the A-buffer.

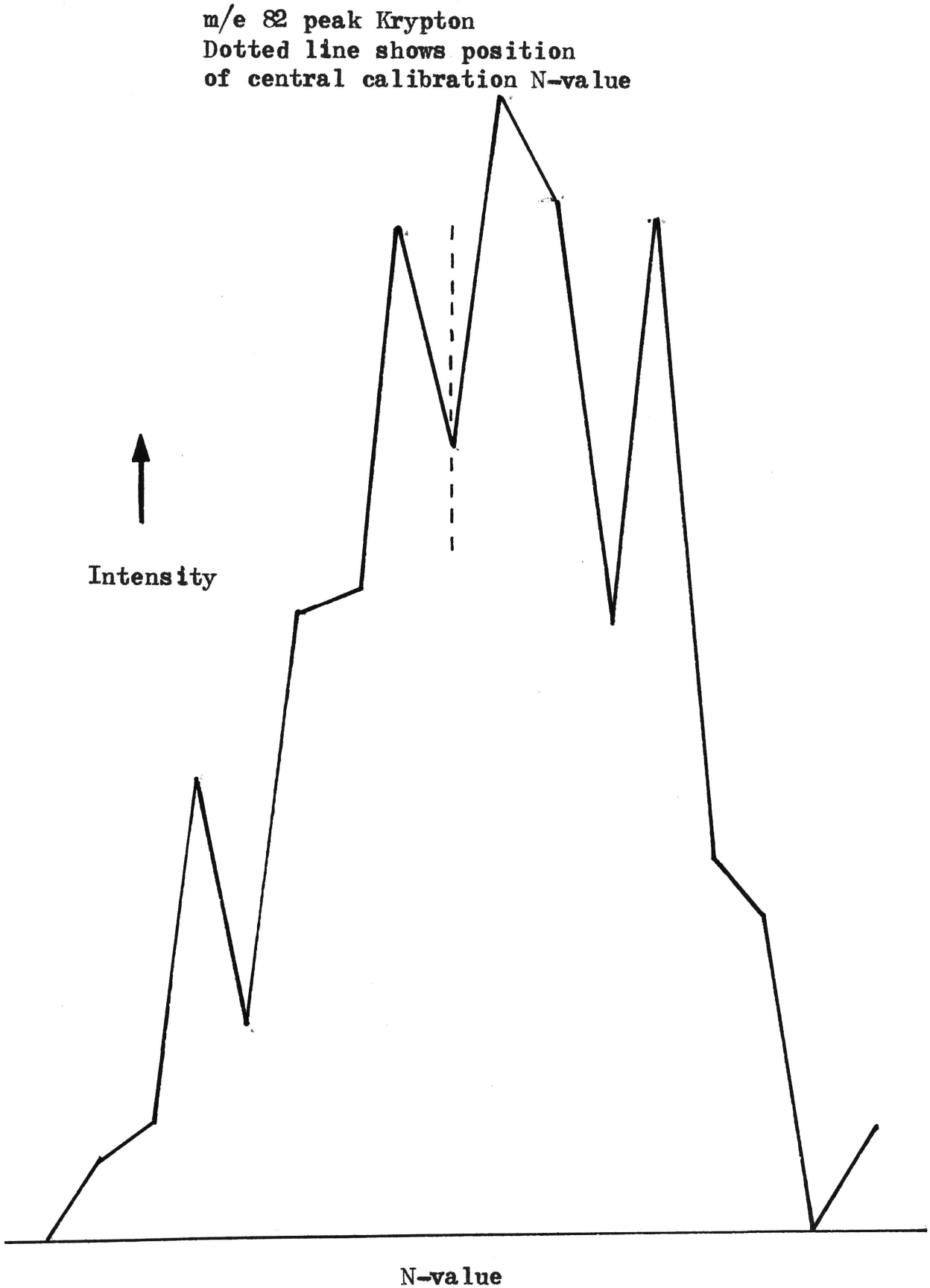
The BACKGROUND segment is used to copy the current contents of the A-buffer into the B-buffer. Thus any spectrum can be stored as a background spectrum for later subtraction from another spectrum.

The DISPLAY segment is the means by which the mass spectra are communicated to the user by way of the teletype or the plotter. Spectra are available as tables of mass and corresponding intensity values as a type-out on the teletype or plotted on an X-Y plotter. Normalised or unnormalised spectra are available, or the largest six peaks in the spectrum can be obtained. Any portion of the normal mass range can be displayed in this way with normalisation always occurring on the largest peak found in the displayed range.

Use of the Quadnova System for Isotope Ratio Measurements

The Quadnova system was essentially designed for qualitative

FIGURE 5


m/e 82 peak Krypton
Dotted line shows position
of central calibration N-value
Intensity
N-value

analysis of mass spectra in order to establish the identity and purity of organic compounds introduced into the system. For this purpose the system has proved highly successful and is regularly used on a routine basis for analysis.

In attempting to perform isotope ratio measurements of the labelled halogenated aromatic derivatives, the Quadnova system was being used in essentially a quantitative manner. It was found that fluctuations in the recorded intensities of mass spectra were of such magnitude that in some cases the pattern of deuterium exchange was distorted, especially with bromine-containing compounds.

These fluctuations may have been caused by one or more of three factors:

(i) a slight non-linearity in the Digital to Analogue (D/A) converter would mean that the intensity sampled was not at the top of the mass peak but on the shoulder. This effect should appear only in particular regions of the mass range.

(ii) Slight thermal drift of the mass spectrometer or interface electronics would also have the same effect but would be evident over the whole mass range.

(iii) Peak splitting has also been seen to occur (figure 5) and would mean that the maximum height of a mass peak will not always occur at the same position, thus leading to fluctuating results. In order to overcome these problems, some changes were made to the Quadnova operating system software, affecting mainly the manner

(iii) in which the programme collects and calculates the intensity at each mass value. The changes were designed to produce an intensity value that was quantitatively more representative of the real intensity value than that produced by the original Quadnova system.

In addition to establishing the exchange pattern and extent of deuteration of organic compounds, a more quantitative system would be useful for isotope ratio determinations in general, for the production of reproducible spectra suitable for matching of unknown spectra (e.g. National Institue of Health Service in U.S.A.) and for the creation of spectra to generate a file of spectra locally.

It has been reported[1] that the inert gases are ideal samples for testing both the precision and accuracy of isotope ratio measurements because their abundances have been reported extremely accurately[60-63] and because there is a single isotopic species per mass, unlike organic compounds where mixtures of carbon, nitrogen, hydrogen and oxygen may contribute to a particular mass. Accordingly Krypton and Xenon were used as reference gases of known isotopic distribution for evaluating the performance of the software.

Methods of Intensity Collection

The modifications made to the Quadnova system consisted of three methods of increasing complexity requiring increasing scan time for the production of quantitative reproducible intensity values.

Method I

This method involves the collection of three intensity values at

each mass value (M-value). To achieve this the N-value calculated from the M-value is passed to the D/A converter and an intensity collected from the A/D converter. The N-value is then decremented by a preset integer amount and another intensity value collected by passing the N-value to the D/A converter. The original N-value is then incremented by the same amount and another intensity value collected. Only the largest of these three intensity values is however stored as the intensity at that mass value.

The rationale for using this technique is that, as described above, a number of factors may be causing the centre of the peak to shift from the point expected from the calibration procedure. Method I was designed to give a greater chance of finding the top of the mass intensity peak than the single collection method of the unmodified Quadnova system. Even if the top of the peak is not found with only three tries the largest of these three should be higher up the peak than with the original system if the peak has drifted somewhat.

This method will also correct for any small differences in mass defect between the compound to be analysed and the reference gas. Most organic compounds have a mass defect of between 0.1 and 0.2 whereas the reference gas has a mass defect closer to zero. Thus the CALIBRATE routine will calibrate the software to nearly integral mass values whereas the mass values of the majority of organic compounds will be slightly higher.

Method I will not overcome the fluctuations due to gross peak splitting however.

Method II

This method involves the collection at each mass value of three or five intensity values located at and on either side of the central N-value followed by the averaging of these intensities to produce a weighted peak average. The variation with three intensity values was an earlier modification, the five intensity value variation was a development from it. The intensity values are collected in a manner similar to that of Method I by incrementing and decrementing the N-value corresponding to the M-value being analysed.

This method is of greater complexity and is slower than Method I. It is designed to produce a weighted composite intensity which will be more representative of the area under the mass peak than a single intensity value. It is in effect similar to a peak area determination yet it is faster than an area determination since only integer arithmetic operations are necessary for averaging.

Method II will tend to overcome fluctuations due to the peak splitting phenomenon since a number of intensities are being collected and a simulated area calculated.

Method III

This method is of greater complexity again and involves the collection at each mass value of five intensity values located at and on either side of the central N-value. From these five intensity values and the baseline width encompassed by the peak a peak area is calculated using the trapezoidal rule. One intensity value is collected two N-values below the N-value corresponding to the M-value being analysed. Four more intensities are then collected by incrementing the N-value by

one. The resultant area is divided by five to produce a number of the order of a single intensity value.

The area under a mass peak should be the best and most reproducible measure of the intensity of a mass peak since the observed fluctuations are such that the peak area appears to remain constant, even with a highly split and fluctuating peak. Any skewness or drift in the mass peak should also be accommodated by the peak area calculation.

Subsidiary Modifications

Since the quantitative techniques of intensity collection described above are all slower scanning than the qualitative Quadnova system, two further modificatinns were adopted to decrease the time spent scanning non-significant peaks. The first modification deals with the actual mass numbers scanned and permits specific ion monitoring. The second provides a threshold value above which intensity a peak is considered to be of significance.

Subroutines QANTM and SPZER are used to permit selective mass entry. The qualitative Quadnova system assumes that a range of contiguous mass values is being scanned and collects an initial and final mass value from the user. Only the intensity is stored at each mass value since its corresponding M-value can be calculated from its position in the buffer and the initial and final mass values. Within this range however a discontinuous mass scan is carried out since intensity values are only collected at integral mass values.

The modification made allows the user to specify individual mass numbers or mass ranges or any combination of these to be scanned. This is achieved by Subroutine QUANTM storing the mass values to be scanned in

the lower half of the B-buffer. The intensities at each mass value are then collected and stored in the top half of the B-buffer. This discontinuous intensity spectrum is converted to one compatible with the DISPLAY segment by the use of subroutine SPZER. This subroutine creates a spectrum from initial to final mass values by filling in an intensity of zero for any mass value that has not been scanned.

The second modification increases the speed of the scan by only applying the quantitative intensity collection routines (Methods I, II and III) to peaks sufficiently above the noise to exceed an automatically set threshold value. To do this an initial intensity value is collected and compared to a threshold. If greater than the threshold value quantitative procedures are adopted, if not then the single intensity is recorded as the intensity at that mass peak. Thus the quantitative methods will appear similar to the qualitative Quadnova system for "noise" peaks, and so speeding up a scan with some "noise" peaks.

The threshold value is calculated by taking an intensity towards the top end of the spectrum (in practice mass 287). This intensity is then multiplied by 1.5, thus ensuring any noise peaks will be below the threshold whilst any peak with a signal to noise ratio of greater than 1.5 will undergo the quantitative intensity collection.

A third subsidiary modification was made to a baseline adjusting technique of the qualitative Quadnova system. The qualitative system contains an inbuilt apparent signal to noise improvement factor in which the baseline is adjusted to the bottom of the intensity spectrum recorded. This is achieved by subtracting the lowest intensity in the spectrum from the rest of the spectrum. However in the quantitative mode a scan may be

made where there is no scanning of noise peaks. Thus if the lowest intensity were to be subtracted from the spectrum a distortion of the intensity values could occur. This is overcome by using the intensity at a mass value where no ions are expected to subtract from the spectrum. In practice the value used is at mass 287.

Evaluation of Modifications to Software

Krypton and Xenon were used as reference gases of known isotopic distribution for primary evaluation of Methods, I, II and III for isotope ratio determinations. Bromobenzene and a deuterated chlorobenzene were used to test the application of the software to the systems which gave rise to the modifications.

The quantitative nature of the Quadnova system was improved by use of the modified procedures developed. The improvement made the system comparable to reported[1,5,17] interactive data systems incorporating a quadrupole mass analyser.

The results are presented as tables of mass and intensity values (in the Experimental section) and mean precision, accuracy and standard deviation from the mean. The mean is given plus or minus one standard deviation from the mean. Precision of the measurements presented is given in terms of the standard deviation from the mean as a percentage of the average value, for the intensities, calculated from eight to ten independent spectra of the gases. Accuracy is expressed as the percentage by which the average abundance differs from the reported literature determinations[60-63] of abundance. These literature figures are not theoretical values but rather the result of accurate high precision

isotope ratio determinations using, with one exception[63], magnetic sector mass spectrometers. Thus the smaller the precision figure the more precise the results, the smaller the standard deviation and the less the fluctuations between spectra. The smaller the absolute accuracy figure the closer are the experimental results to those of the literature. Literature relative abundances have been converted (Tables 24 and 25) to normalised abundances with the largest value set to 1000. With the spectra presented,mean, standard deviation, accuracy and precision are not given for the ion normalised on (84 for Krypton, 132 for Xenon) since its value is always 1000.

TABLE 24

Krypton relative abundance[62]

Mass	Relative abund.	Normalised Abund.
78	0.355 ± .001	6
80	2.256 ± .007	40
82	11.553 ± .009	203
83	11.536 ± .009	202
84	56.982 ± .01	1000
86	17.318 ± .009	304

TABLE 25

Xenon relative abundance[63]

Mass	Relative abund.	Normalised Abund.
124	0.097 ± .001	4
126	0.090 ± .001	3
128	1.927 ± .003	72
129	26.49 ± .04	986
130	4.078 ± .005	152
131	21.21 ± .03	788
132	26.86 ± .04	1000
134	10.41 ± .02	388
136	8.83 ± .01	329

For Krypton the results obtained (Table 26) show that in general Method III gives the most precise and accurate results (Table 27). However all the quantitative modifications are an improvement in precision and accuracy of Krypton analyses compared to the qualitative system.

Isotope mass discrimination is evident with the qualitative system, and to a lesser extent with Method I, since some mass to charge ratios are more accurately (less absolute difference between mean and literature results) analysed than others. Ion source geometry has been reported[62,64] to have the effect of isotope mass discrimination but other factors, such as relative ionization efficiency or isotope fractionation during separation of the inert gases from the atmosphere, may also be operative. This isotope mass discrimination is not as evident with the latter quantitative modifications.

TABLE 26

Summary, Isotope Ratio Analyses of Krypton

m/e	Literature value	Qualitative System	Method I	Method II	Method III
78	6	7.6 ± 0.9	6.3 ± 0.5	6.1 ± 0.7	6.1 ± 0.6
80	40	39.0 ± 1.0	40.4 ± 0.5	38.6 ± 2.1	40.4 ± 0.6
82	203	240.1 ± 4.2	203.4 ± 2.8	208 ± 10.0	204.6 ± 1.7
83	202	180.3 ± 1.9	200.0 ± 2.4	200 ± 11.4	202.4 ± 1.6
84	1000	1000	1000	1000	1000
86	304	238 ± 6.4	296.4 ± 4.2	276 ± 13.6	298.5 ± 1.2

TABLE 27

Precision and Accuracy of Krypton Analyses

m/e	Qualitative System		Method I		Method II		Method III	
	Precision	Accuracy	Precision	Accuracy	Precision	Accuracy	Precision	Accuracy
78	11.7	+22.3	7.9	+2.9	1.1	+2.3	9.8	+1.7
80	2.5	0	1.2	+1.4	5.4	-3.5	1.5	+1.0
82	1.8	+18.2	1.4	+0.1	4.8	+2.5	0.8	+0.7
83	1.1	-11.3	1.2	-1.5	5.7	-1.7	0.8	+0.5
84	-	-	-	-	-	-	-	-
86	2.7	-21.9	1.4	-2.9	4.9	-9.5	0.4	-2.1

TABLE 28

Summary, Isotope Ratio Analyses of Xenon

m/e	Literature value	Qualitative System	Method I	Method II	Method III
128	72	72.0 ± 1.3	83.1 ± 2.7	73.8 ± 1.2	70.7 ± 1.0
129	986	1087.1 ± 4.5	965 ± 16.2	1007 ± 9.3	993 ± 12.2
130	152	154.7 ± 2.3	162.8 ± 3.9	154.7 ± 1.4	154.1 ± 1.2
131	788	700.7 ± 4.2	786 ± 17.2	793 ± 5.4	813.5 ± 7.2
132	1000	1000	1000	1000	1000
133	388	388.6 ± 5.5	393 ± 6.6	388.5 ± 3.5	384.3 ± 4.6
136	329	332.1 ± 4.7	334.7 ± 4.5	328.9 ± 3.4	310.7 ± 3.9

TABLE 29

Precision and Accuracy of Xenon Analyses

m/e	Qualitative System		Method I		Method II		Method III	
	Precision	Accuracy	Precision	Accuracy	Precision	Accuracy	Precision	Accuracy
128	1.7	0	3.2	+16.9	1.6	+4.2	1.5	-1.4
129	0.4	+10.2	1.7	- 1.8	0.9	+2.3	1.2	+0.9
130	1.4	+ 2.0	2.4	+ 6.7	0.9	+1.7	0.8	+1.3
131	0.6	-10.1	2.2	- 0.4	0.7	+0.7	0.9	+3.2
132	-	-	-	-	-	-	-	-
133	1.4	+ 0.2	1.7	+ 1.3	0.9	+0.1	1.2	+1.0
136	1.4	+ 0.6	1.3	+ 1.4	0.1	-0.3	1.3	-6.1

Method II does not appear to offer (Tables 26 and 27) any further improvement over the results obtained with Method I, although Method II is more recise and accurate than the qualitative system. This lack of significant improvement compared to Method I may be due to the "weighting" process, the weights applied to each intensity may not be in the correct ratio to simulate the peak shape or the weighting of the intensity from the central N-value may be too large.

The results for Method III (Tables 26 and 27) for analysis of Krypton show further improvement in precision and accuracy compared to Methods I and II. This is a reflection of smaller fluctuations and the more reproducible spectra obtained by analysis with Method III.

The Xenon analyses (Table 28) show again that Method III is the most precise (Table 29) and in general most accurate of the methods evaluated. Method I is more accurate but slightly less precise than the qualitative system, but this may in part be due to baseline problems, discussed later.

The results for Xenon analysis by Method II (Table 28) show an improvement in precision and accuracy (Table 29) relative to the unmodified Quadnova system.

Method III shows little improvement over Method II, this being possibly due to baseline problems observed with Method III, since the intensity is now an area derived number, whereas the noise subtraction value is calculated as a "single shot" intensity.

This factor means that some distortion of the peaks may arise. The effect of "electronic noise" on the spectrum of Krypton (Table 30) shows that the actual level of noise suppression has an effect even on a

TABLE 30

Krypton, Effect of noise level on intensity results (Method I)

	MASS			
	82	83	84	86
unnormalised	2142	2106	10039	3112
normalised	213	210	1000	310
noise level 170 subtracted then normalised	200	196	1000	298
noise level 160 subtracted then normalised	201	197	1000	299
noise level 155 subtracted then normalised	199	195	1000	296
noise level 150 subtracted then normalised	199	196	1000	297

normalised spectrum. Further investigation is being carried out in this field.

Method I contains a decrementing factor which is used to determine the change in N-values when collecting the intensity values. This decrement may be altered and the effects of this alteration with the range zero to five have been evaluated (Table 31). From the results it is apparent that decrement 2 provides the most precise and accurate determination of intensity. This indicates that the maximum shift in mass peaks is $\pm$ 0.1 atomic mass units since two N-values are one tenth a.m.u. and Method I is designed to find the top of the mass peak.

TABLE 31

Effect of changing decrement on intensity of m/e 158 of bromobenzene (literature value 977) compared to m/e 156 (set to 1000).

	Decrement					
	0	1	2	3	4	5
Mean	950	951	976.2	944	937	933
Std. Dev.	7.0	13.2	2.9	13.9	18.5	18.5
Precision %	0.7	1.4	0.3	1.5	2.1	2.0
Accuracy %	2.8	2.7	0.1	3.4	4.2	4.6

The pre-amplifier used to boost the signal from the quadrupole mass analyser to the electronics contains a number of gain settings. The C and D scales are those most commonly used, D scale for most qualitative analysis. The results obtained with Method I, C and D Scales (Table 33)

TABLE 33

Krypton analysis C and D Scale Method I

	m/c	Mean	Std. dev.	Precision %	Accuracy %
C Scale	82	286.2	1.8	0.6	40.9
	83	296.6	3.0	0.9	45.8
	86	374.6	4.2	1.0	22.6
D Scale	82	208.8	3.7	1.8	2.5
	83	207.6	3.9	1.9	2.0
	86	307.4	2.0	0.7	0.7

show that D Scale is the more accurate. This scale was used for all isotope ratio determinations carried out.

It is important to note that determination, with high precision and accuracy, of isotope ratios by direct analysis may be considered a unique type of analysis, pursued for its own value. It cannot in general be achieved simultaneously with the accumulation of wide scan range GC-Ms data. The attainment of a great deal of structural information is achieved only at the sacrifice of precision[1,45] and vice versa, although favourable cases may exist where precision and structural information may be determined concurrently.

Operational considerations[67] of the mass spectrometer bear a good deal of weight in the attainment of high precision. Memory effects, background cleanliness and resolution are the major operational factors to be considered. Samples in an isotope series should be analysed in increasing order of enrichment to minimize memory effects,[45,65] due to adsorption on the internal surfaces of the system and the system should be flushed with the sample to be analysed if at all possible. Background due to traces of previously analysed samples of other material may distort observed isotope ratios. After system start-up background due to condensation on the filament or GC column bleed may be observed but this can be eliminated by allowing the filament to 'warm up' before analysis. Mass resolution also plays an important part in the attainment of high precision isotope ratio determinations, since it affects peak shape. The closer the shape of a mass peak to a "rounded-top" the better[1] the precision that can be obtained. However this method of tuning decreases the sensitivity so a compromise of 8-10% "valley" between adjacent peaks was used since any higher "valley" will lead to peak tailing, adding to the intensity of the next peak and giving poor accuracy, whilst a lower

resolution produces sharp peaks more likely to drift off calibration.

In order to determine whether the dynamic sample pressure fluctuations observed with the effluent from a G.C column could be handled by the quantitative system and to assess the application of Method I to the systems which gave rise to the modifications, a slightly deuterated chlorobenzene was analysed by G.C. injection. The results (Table 34) show that precision is at least as good as that obtained from

TABLE 34

Analysis of slightly deuterated chlorobenzene (normalised on m/e 112)

	m/e 112	m/e 114 (exp)	m/e 114 (lit)
Mean	1000	345.5	327
Standard dev.	-	1.5	-
Precision	-	0.4	-

liquid (gas) injection of the inert gases. An accuracy figure would have no significance since the substance is slightly deuterated.

Thus the modifications made to produce a more quantitative Quadnova system appear to be successful, Method III in general being the most satisfactory. However Method I should not be overlooked, as it has greater simplicity and is therefore faster scanning than Method III.

EXPERIMENTAL

EXCHANGE REACTION PROCEDURES

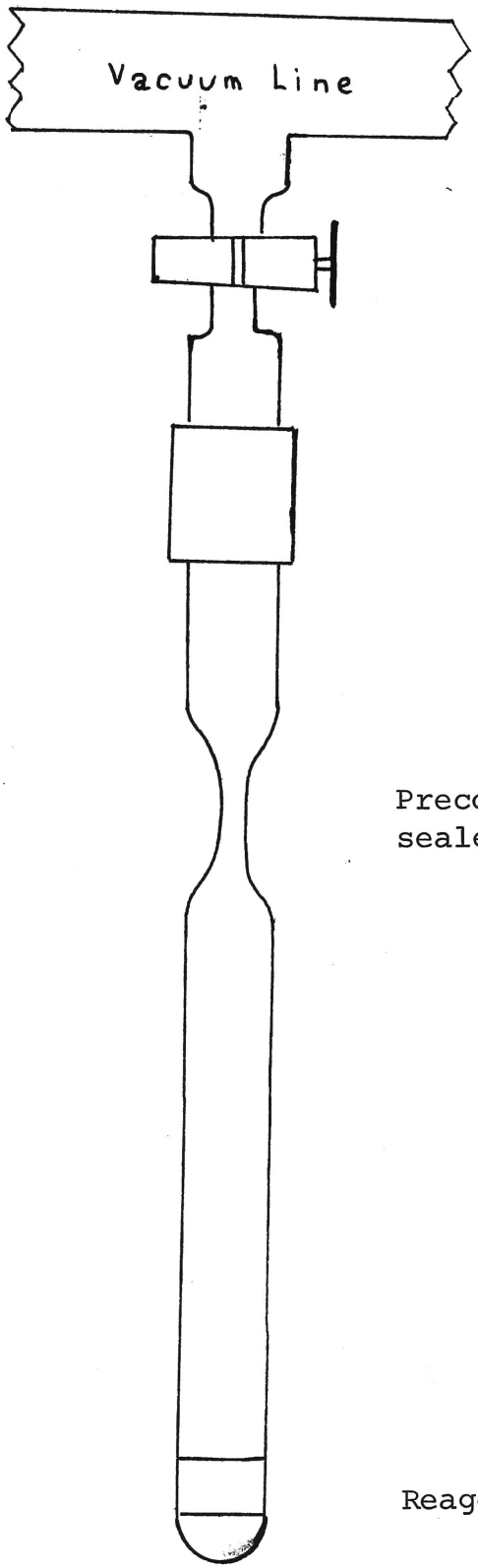
In a typical experiment sodium borohydride (200 mg) was added slowly to a solution at room temperature of the metal salt, containing the equivalent of 40 mg of the metal. A black suspension was formed as the metal was reduced. Hydrolysis of the excess borohydride was completed by warming the suspension of the reduced catalyst to 70°C and keeping it at that temperature for ten minutes. With iridium, no reduction to the metal from the salt occurred until the solution was warmed to 70°C.

The suspension was then centrifuged and the supernatant liquid decanted. The reduced catalyst was washed free from excess salts with water, centrifuging and decanting the supernatant after each washing. The metal (10 mg) was then placed into the pyrex glass reaction tube (figure 6) together with the appropriate amount of the organic substance to be exchanged and one ml of deuterium oxide. The reaction mixture was frozen in an alcohol-dry ice slush bath and evacuated to a pressure of 0.010 torr or less. The sample was then allowed to warm slowly to room temperature under vacuum, frozen again and any gases remaining again evacuated. This procedure is designed to remove any dissolved gases from the reaction system. The sample tube was then sealed under vacuum at the point where it had been preconstricted.

The reaction was then carried out at elevated temperature and upon completion of the standard reaction time (1 week) the tube was broken open and the organic layer separated for subsequent mass spectrometric analysis with the Quadnova system.

FIGURE 6

REACTION TUBE FOR EXCHANGE REACTION



Due to fluctuations observed between consecutive mass spectra with the original Quadnova system for deuterium analysis particularly of bromine containing compounds, the bromobenzenes were reanalysed with method I of the quantitative modification to the software. This resulted in more reproducible spectra and the results presented for bromobenzenes (Tables 3 and 4) are those from this quantitative procedure.

GENERAL EXPERIMENTAL CONDITIONS

The mass spectroscopic determinations were carried out at low ionizing voltage to minimize fragmentation of the molecules during analysis. Samples were injected into the EAI Quad. 300 mass spectrometer via the liquid injection system. The instrument was flushed twice with the sample to be analysed to minimize any memory effects. The inert gases were introduced via a gas tight syringe into the liquid injection system.

Instrument conditions used for isotope ratio determinations were as follows:

Ionization Chamber Temperature		250°C
Emission current		4 amps
Ion energy		5 volts
Ionizing voltage	(Deuterium analysis)	12 eV
	(Kr and Xe analysis)	50 eV

Gas liquid chromatography separations were performed on two instruments, a CARLE Model 8000 basic gas chromatograph, fitted with a six foot 1/8 inch outside diameter stainless steel column containing 8% carbowax 1540 on ANAKROM ABS solid support, was used for isothermal work at 150°C with a thermal conductivity detector. A PACKARD model 419

Becker Gas Chromatograph fitted with an eight foot by 1/8 inch outside diameter stainless steel column of 3% Apiezon N on Gas Chrom Q with a flame ionization detector was used for temperature programmed G.C. runs.

An Aerograph 300C gas chromatograph was used to perform G.C.-M.S. analyses and was fitted with a six foot 1/8 inch outside diameter stainless steel column of Apiezon N.

Proton Magnetic Resonance measurements were carried out on an Hitachi-Perkin-Elmer R24A and a Varian A60 spectrometers operating at 60 Mc/s and using tetramethylsilane (T.M.S.) as an internal reference. For signal area determinations, the average of five pmr spectrum integrals was taken and the method described earlier (page 41) used to calculate the deuterium distribution ratios.

Calculation of Isotopic Enrichment

In the mass spectra of all compounds containing carbon there are isotope peaks, due to the presence of naturally occurring isotopes, particularly carbon - 13 and deuterium, which is observed one atomic mass unit above each molecular or fragment ion. For compounds containing chlorine and bromine (which both have second isotopes of high natural abundance) the isotope peak occurs two a.m.u. above the parent peak.

When such compounds are deuterated the spectrum is changed by isotopic enrichment with deuterium. To calculate the percentage overall deuterium incorporation into the molecule some correction must be made for both natural carbon 13, deuterium and halogens. The isotope correction formula for carbon-13 is as follows:

$$\frac{\text{Height of m + 1 peak}}{\text{Intensity of m peak}} = \frac{n.X.100}{1-X}$$

$$= 1.11n\%$$

where m = parent ion peak

m + 1 = isotope peak one mass unit from the parent ion peak

n = number of carbon atoms in molecule

X = fraction of carbon occurring as carbon-13

When this formula is used the observed intensities are corrected to produce an intensity due solely to deuterium enrichment. Thus for a molecular ion of composition C_6H_6 the observed intensity I_{M+1} at the ion (M + 1) is corrected to give an intensity I_c by the use of the formula as illustrated below:

$$I_c = I_{M+1} - 6 \times 0.11 \times I_M$$

Similarly the intensity at the ion M + 2 will need to be corrected for a Cl.37 or Br.81 contribution if these species are present. Thus, for example, with C_6H_5Br

$$I_{M+2} = I_{M+2} - 6 \times 0.11 \times I_{M+1} - 0.9775 \times I_M$$

For multiple exchange of deuterium into a fragment the average deuterium content, , is calculated from the following equation using the corrected intensities as calculated above

$$= \frac{1}{n} \sum_{i=1}^{n} idi$$

where di is the percentage of aromatic molecules (of n hydrogen atoms) containing i deuterium atoms.

Isotope Correction Program

The formulae described above were used in an istope correction program written in Basic for use on the Chemistry Department's Nova 1220 mini-computer. The program identifies itself when used and requires the

input of the spectrum number and peak heights in order from D_0 upwards. An identifying heading is printed for each analysis together with the spectrum number. The extent of deuterium incorporation in the molecule is calculated by the program.

The program as shown can be used for isotope correction for bromine containing compounds, for chlorine containing compounds statement 25 is altered to read:

```
LET  F  =  0.3239 *C1
```

The program listing appears in the appendix.

Inert Gas Analyses

The inert gases Krypton and Zenon were used as test samples to evaluate the program changes. The spectra were recorded using conditions as noted earlier and are presented as tables of mass to charge ratio (abbreviated to "mass") and intensity values. The mean is given plus or minus one standard deviation, calculated[65,66] from the formula:

$$S = \sqrt{\frac{\overline{X} - X)^2}{N-1}}$$

Where $\overline{X}$ is the mean of the intensity values for that particular mass to charge ratio.

KRYPTON: Isotope ratio determination results.

Spectra are normalised to mass 84, which has been set to 1000.

Krypton - Qualitative Quadnova System

	MASS				
	78	80	82	83	86
INTENSITY	8	40	250	179	229
	8	38	242	177	229
	6	37	240	178	233
	8	40	241	181	239
	8	39	236	181	238
	7	39	237	181	242
	7	39	237	182	241
	9	40	240	183	246
	7	39	238	181	245
MEAN	7.6 ± 0.8	39.0 ± 1.0	240.1 ± 4.2	180.3 ± 1.9	238.0 ± 6.4

Krypton - Method I

MASS

	78	80	82	83	86
INTENSITY	6	41	206	201	295
	7	41	201	196	298
	7	41	203	199	296
	7	40	198	197	286
	6	40	205	202	301
	6	40	202	199	296
	6	41	205	203	296
	6	40	204	201	298
	6	40	207	202	302
MEAN	6.3 ± 0.5	40.4 ± 0.5	203.4 ± 2.8	200.0 ± 2.4	296.4 ± 4.2

Krypton - Method II (five peaks)

MASS

	82	83	86
INTENSITY	198	202	251
	192	189	255
	206	207	283
	207	201	269
	228	225	301
	210	203	272
	217	205	278
	217	203	279
	221	207	281
	203	187	268
	204	187	275
	207	194	290
	209	196	294
	195	187	269
MEAN	208 ± 10.0	200 ± 11.4	276 ± 13.6

Krypton - Method III

	MASS 82	83	86
INTENSITY	210	223	300
	204	199	297
	203	199	296
	204	199	298
	204	201	297
	204	201	299
	204	201	299
	203	200	300
	205	202	301
	206	202	209
	204	201	299
	204	201	298
	205	203	297
	204	202	299
MEAN	204.6 ± 1.7	202.4 ± 1.6	298.5 ± 1.2

Krypton - Method II (three peaks)

	MASS 82	83	86
INTENSITY	216	211	269
	219	208	267
	214	206	266
	218	211	270
	211	206	262
	213	203	261
	215	204	261
	215	203	261
	214	204	262
	214	204	259
	216.7 ± 3.6	206.0 ± 3.1	263.8 ± 3.3

Method II (three peaks)

Integration gain : L integration time : 100 milliseconds

	MASS		
	82	83	86
INTENSITY	216	173	270
	216	183	275
	212	183	279
	210	185	274
	214	189	283
	213	188	279
	217	192	287
	215	192	285
	212	188	279
	217	192	286
MEAN	214.2 ± 2.4	186.5 ± 5.8	280 ± 5.6

Method II (three peaks)

Integration gain : M integration : 5 milliseconds

	MASS		
	82	83	86
INTENSITY	231	158	261
	215	166	255
	225	177	269
	219	181	275
	219	181	254
	226	187	262
	219	177	261
	206	170	258
	210	174	264
	198	167	256
	219	183	254
	226	186	279
	217	183	264
MEAN	218 ± 8.9	176 ± 8.7	263 ± 7.8

Krypton - Method I - C and D Gain Settings

C Setting

	MASS		
	82	83	86
INTENSITY	285	301	380
	285	398	376
	285	296	372
	287	294	369
	289	294	376
MEAN	286.2 ± 1.8	296.6 ± 2.9	374.6 ± 4.2

D Setting

	MASS		
	82	83	86
INTENSITY	213	210	310
	208	204	308
	203	204	303
	210	207	306
	210	213	310
MEAN	208.8 ± 3.7	207.6 ± 3.9	307.4 ± 2.0

XENON: Isotope Ratio Determination Results

Spectra were recorded with instrumental conditions as previously noted. All spectra are normalised to mass 132 which has been in all cases set equal to 1000.

Xenon - qualitative Quadnova System

	MASS					
	128	129	130	131	134	136
INTENSITY	73	1090	154	700	388	327
	71	1075	153	701	383	326
	72	1065	152	695	383	327
	70	1066	151	697	382	329
	72	1071	152	699	384	330
	70	1082	154	702	383	329
	71	1077	154	702	385	333
	71	1078	154	702	384	332
	72	1101	156	708	391	333
	74	1097	156	709	391	337
	74	1099	157	705	396	338
	73	1095	157	700	393	336
	73	1096	157	696	394	338
	73	1111	159	696	397	337
	72	1104	155	698	395	330
MEAN	72.0 ± 1.3	1087.1 ± 4.51	154.7 ± 2.26	700.7 ± 4.2	388.6 ± 5.5	332.1 ± 4.7

Xenon - Method I

	MASS					
	128	129	130	131	133	136
INTENSITY	87	957	166	782	401	340
	86	942	168	797	397	345
	84	965	165	803	400	339
	85	982	166	803	394	344
	80	990	166	808	399	340
	81	939	157	762	386	329
	81	968	159	773	388	325
	80	978	159	771	391	328
	84	967	159	772	383	322
MEAN	83.1 ± 2.7	965 ± 16.2	162.8 ± 3.9	786 ± 17	393 ± 6.6	334.7 ± 4.5

Xenon - Method II (five peaks)

	MASS					
	128	129	130	131	133	136
INTENSITY	74	1011	154	789	380	324
	71	985	151	782	385	323
	73	1013	156	797	385	324
	73	1012	156	798	390	330
	74	1002	155	789	390	329
	74	1000	154	788	389	330
	75	1023	157	798	394	335
	75	1007	155	792	391	332
	74	1015	156	793	393	329
	75	1008	154	790	385	326
	75	1014	155	802	390	332
	74	1004	155	799	388	329
	75	1010	155	791	388	328
	73	1005	154	792	392	334
	72	991	153	801	388	328
MEAN	73.8 ± 1.2	1007 ± 9.3	154.7 ± 1.4	793 ± 5.4	388.5 ± 3.5	328.9 ± 3.4

Xenon - Method III

	MASS					
	128	129	130	131	133	136
INTENSITY	73	1009	154	812	382	307
	70	1000	156	830	393	318
	70	1020	156	827	395	317
	70	1003	154	818	391	318
	70	991	152	809	381	310
	70	1000	153	807	381	310
	70	994	153	807	381	309
	70	979	153	808	381	305
	70	977	153	806	381	307
	72	998	156	821	387	312
	71	984	154	814	382	309
	72	988	155	812	381	309
	71	988	155	812	383	312
	72	981	154	812	382	310
	70	976	154	807	383	307
MEAN	70.7 ± 1.0	993 ± 12.2	154.1 ± 1.2	814 ± 7.2	384.3 ± 4.6	310.7 ± 3.9

Bromobenzene

Spectra normalised on mass 156 (set to 1000).

All figures in tables are relative intensities for mass 158.

The literature[60] isotope Br81 natural abundance is 977.5 (relative to Br79 set to 1000).

	DECREMENT					
	0	1	2	3	4	5
INTENSITY	941	967	977	938	961	885
	943	974	978	940	956	931
	954	941	972	956	941	930
	950	948	975	967	941	931
	957	931	980	944	940	938
	953	956	979	950	952	946
	945	938	975	949	901	930
	960	950	975	914	950	949
	943	959	972	937	898	945
	959	948	979	948	925	945
MEAN	950 ± 7.0	951 ± 13.2	976.2 ± 2.9	944 ± 13.9	937 ± 18.5	933 ± 18.5

Slightly Deuterated Chlorobenzene

G.C. injection at 100°C isothermal

Spectra normalised to mass 112 (set to 1000), the intensities in the table are of mass 114 relative to mass 112. The chlorobenzene was slightly deuterated (0.9%)

Intensity
344
346
348
347
343
344
346
MEAN 345.5 ± 1.5

APPENDIX

ISOTOPE CORRECTION PROGRAM

```
  PRINT
  PRINT "GRP ISOTOPE CORRECTION PROG."
0  DIM A[20]
5  READ C,H,C1
0  DATA  6, 5, 1
5  LET F=.9775*C1
5  LET G=.0125*C
3  PRINT
0  PRINT "SPECTRUM NUMBER =";
0  INPUT S1
3  PRINT
5  PRINT "INPUT PEAK HEIGHTS"
0  FOR I= 0 TO H
5    INPUT A[I]
0  NEXT I
5  PRINT
5  PRINT
5  FOR I=1 TO H
0    LET A[I]=A[I]-G*A[I-1]
5    IF A[I]>= 0 GOTO  85
0    LET A[I]= 0
5  NEXT I
0  FOR I=2 TO H
     LET A[I]=A[I]-F*A[I-2]
     IF A[I]>= 0 GOTO  95
     LET A[I]= 0
5  NEXT I
00  LET S=A[ 0]
01  LET M=A[ 0]*H
02  LET N= 0
05  FOR I=1 TO H
 0    LET S=S+A[I]
 5    LET M=M+A[I]*(H-I)
 0    LET N=N+A[I]*I
 0  NEXT I
 5  LET N=N/S
 0  LET M=M/S
 5  LET C=N/(N+M)*100
 0  FOR I= 0 TO H
 5    LET A[I]=A[I]/S*100
 0  NEXT I
 5  PRINT "TEST"
 8  FOR I= 0 TO H
 9    IF A[I]>=.1 GOTO  171
 0    LET A[I]= 0
 1  NEXT I
 2  PRINT
 3  PRINT "SPECTRUM NUMBER";
 4  PRINT S1
```

```
78  PRINT
79  PRINT
80  FOR I= 0 TO H STEP 3
85    LET I1=I+1
90    LET I2=I+2
95    PRINT "PEAK",I,I1,I2
00    PRINT
05    PRINT "CORR PC ",A[I],A[I1],A[I2]
10    PRINT
15    PRINT
20  NEXT I
25  PRINT "OVERALL PERCENTAGE DEUTERIUM INCORPORATED =";
30  PRINT C
35  END
```

METHOD I

Description of Program Modifications

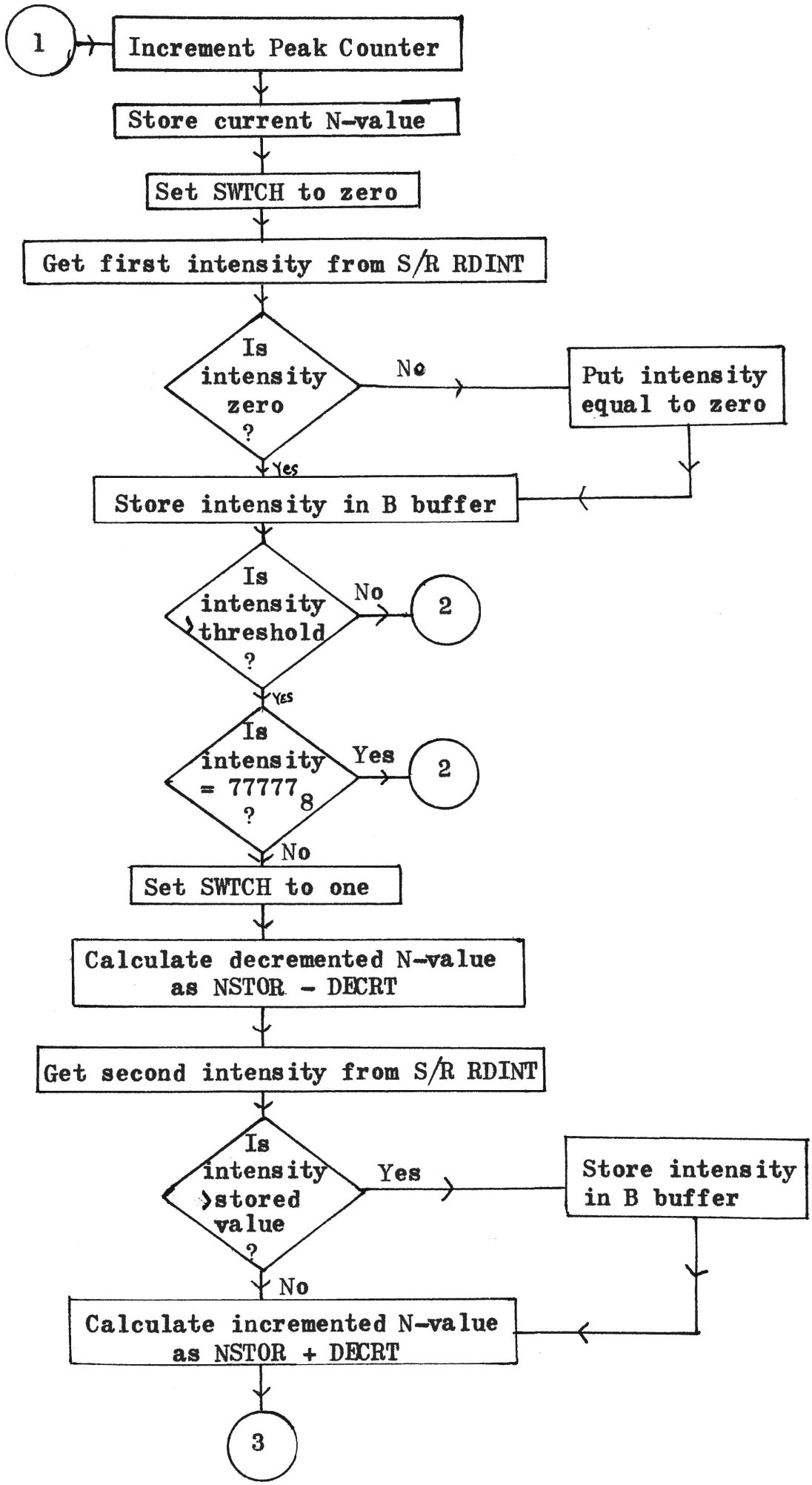
The modifications made to the SCAN segment for method one commence at NLOOP. At NLOOP the peak counter (PCNT) is incremented and then the current N-value stored in (NSTOR). SWTCH is then set to one and a jump made to subroutine RDINT to collect the intensity value. A check is made to see if this intensity value is greater than zero, if not it is replaced by zero. The intensity is then stored in the B-buffer and a check is then made to see if the intensity is greater than the threshold. If not the program exits and goes on to collect the intensity for the next mass number. If the intensity is equal to the maximum ($77777_8 = 32,767_{10}$) then again the program goes on to collection of the intensity of the next mass value. These two checks are designed to speed up the scan so that quantitative analysis is not carried out on peaks below the threshold or at overload. If a "normal"peak whose intensity is between the threshold and overload limits is encountered then the program enters the quantitative routine. SWTCH is set to one to stop RDINT calculating a false N-value. The decremented N-value is calculated by subtracting a preset decrement (DECRT) from the current N-value (in NSTOR). An intensity corresponding to this N-value is then collected by a jump to subroutine RDINT. This intensity is compared with that in the buffer (from the first intensity collection) and if larger is stored in the buffer replacing the previous value. The incremented N-value is then obtained by addition of the preset decrement (DECRT) to the central N-value (NSTOR). An intensity corresponding to this N-value is then collected by a jump to subroutine RDINT. This intensity is compared with the intensity stored in the

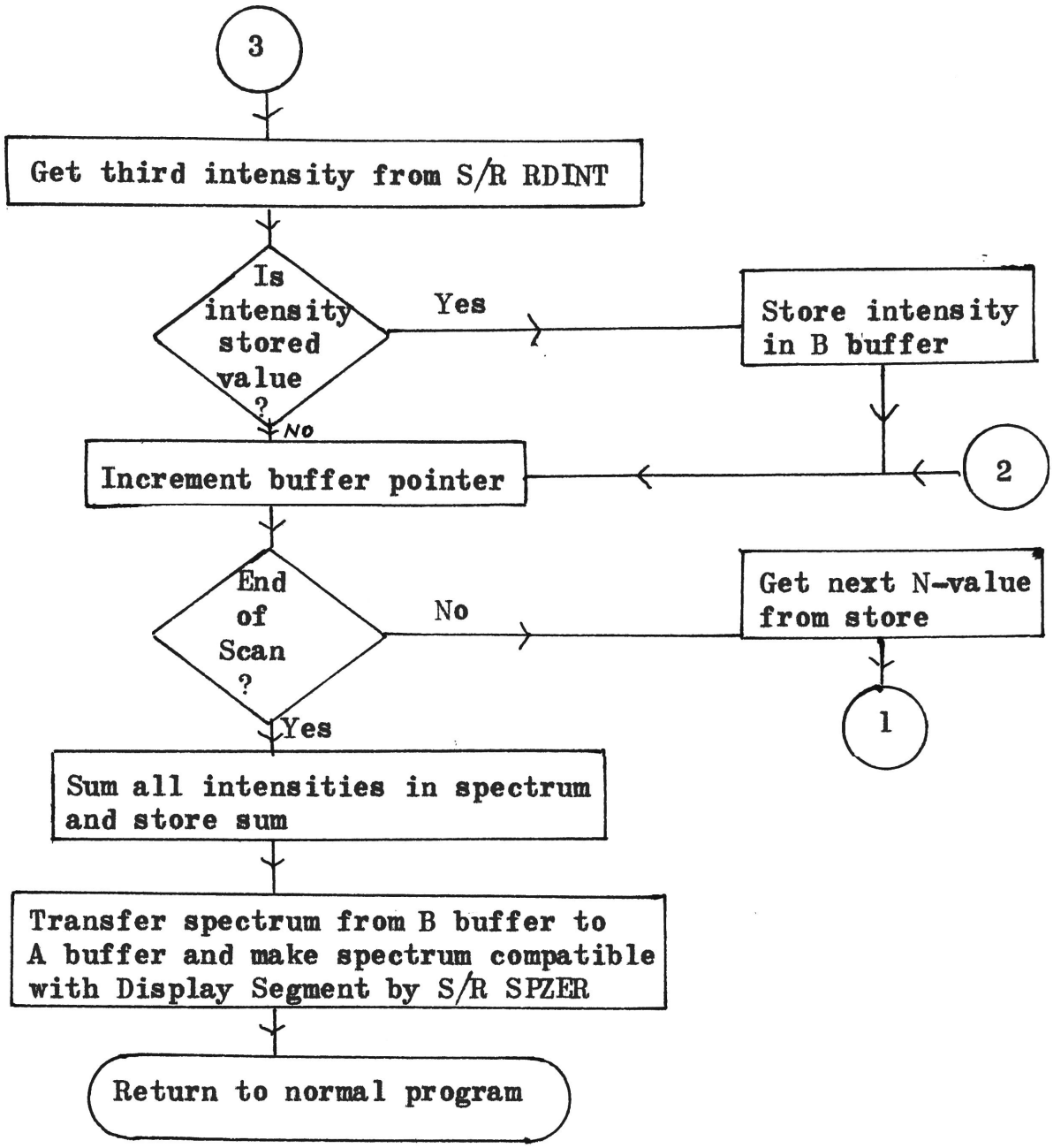
buffer (the largest of the first two collected intensities) and if larger is stored in the buffer replacing the previous value. The next section (NL1) checks to see if the scan is complete. This is achieved by incrementing the buffer pointer in case another intensity has to be collected and then comparing the running total of mass values whose intensities have been collected in this scan (MCNT) to the total number to be collected (PCNT). If this is not the end of the scan the next N-value (calculated by subroutine RDINT during a hardware delay) is loaded into AC-0 and a return made to NLOOP to continue the scan. If the scan is complete the buffer pointer is loaded into accumulator two and the number of peaks (PCNT) loaded into a counter (CNT) via AC-1. The next loop (NOISE) is used for noise subtraction in the original Quadnova. However with method one the noise subtraction feature has been deleted and the loop sums the peak intensities, as a double precision number, to produce an indication of the total ion current. After this loop a jump to subroutine SPZER is made to convert a possibly discontinuous spectrum in the B-buffer into a continuous spectrum in the A-buffer, suitable for use with the Display segment. The remaining sections of the SCAN segment remain unaltered except for a small modification to subroutine RDINT. This modification was made since previously every time the subroutine was called a new N-value was calculated during a hardware delay. However this was not desired now since each call of subroutine RDINT will not necessarily be for a different mass value. The actual modification consists of introducing the following statements into subroutine RDINT so as to skip the statement which increments the mass value buffer pointer.

```
LDA     0, SWTCH
SKZER   0,0
JMP     +2
```

The dummy calculation of an N-value from an M-value was retained to allow the hardware setting time to remain as in the qualitative Quadnova system.

FLOW CHART OF METHOD I




3
Get third intensity from S/R RDINT
Is intensity stored value ?
Yes
Store intensity in B buffer
No
Increment buffer pointer
2
End of Scan ?
No
Get next N-value from store
1
Yes
Sum all intensities in spectrum and store sum
Transfer spectrum from B buffer to A buffer and make spectrum compatible with Display Segment by S/R SPZER
Return to normal program

```
---
↑↑↑
  03747  010137  NSCAN:  ISZ     SCNT
  03750  006000          JSR     @PABCL
  03751  102440          SUBO    0,0
  03752  040133          STA     0,OVCNT
  03753  040113          STA     0,PCNT
  03754  040000          STA     0,BCNT
  03755  010000          ISZ     BCNT
  03756  024227          LDA     1,CBUFF
  03757  044226          STA     1,MBUFF
  03760  022226          LDA     0,@MBUFF
  03761  024232          LDA     1,BBUFF
  03762  044231          STA     1,ASET
  03763  006112          JSR     @MCONV
  03764  010113  NLOOP:  ISZ     PCNT
  03765  040530          STA     0,NSTOR
  03766  126400          SUB     1,1
  03767  044222          STA     1,SWTCH
  03770  006114          JSR     @INTRD
  03771  100513          SKGTZ   0,0
  03772  121000          MOV     1,0
  03773  042231          STA     0,@ASET
  03774  024675          LDA     1,THRES
  03775  106513          SKGT    0,1
  03776  000424          JMP     NL1
  03777  024517          LDA     1,MAXM
  04000  122415          SKNEQ   1,0
  04001  000421          JMP     NL1
  04002  126520          SUBZL   1,1
  04003  044222          STA     1,SWTCH
  04004  024223          LDA     1,DECRT
  04005  020510          LDA     0,NSTOR
  04006  122400          SUB     1,0
  04007  006114          JSR     @INTRD
  04010  026231          LDA     1,@ASET
  04011  122513          SKGT    1,0
  04012  042231          STA     0,@ASET
  04013  020502          LDA     0,NSTOR
  04014  024223          LDA     1,DECRT
  04015  123000          ADD     1,0
  04016  006114          JSR     @INTRD
  04017  026231          LDA     1,@ASET
  04020  122513          SKGT    1,0
  04021  042231          STA     0,@ASET
  04022  010231  NL1:    ISZ     ASET
  04023  020113          LDA     0,PCNT
  04024  024230          LDA     1,MCNT
  04025  122513          SKGT    1,0
  04026  000403          JMP     .+3
  04027  020004          LDA     0,NVAL
```

```
04030 000734            JMP     NLOOP
04031 030232            LDA     2,BBUFF
04032 024113            LDA     1,PCNT
04033 044000            STA     1,CNT
04034 025000 NOISE:     LDA     1,0,2
04035 151400            INC     2,2
04036 102400            SUB     0,0
04037 006105            JSR     @DSUM
04040 000115            TOTIC
04041 040115            STA     0,TOTIC
04042 044116            STA     1,TOTIC+1
04043 014000            DSZ     CNT
04044 000770            JMP     NOISE
04045 006225            JSR     @PSPZR
04046 020115 SNCAL:     LDA     0,TOTIC
04047 024116            LDA     1,TOTIC+1
04050 030113            LDA     2,PCNT
04051 006123            JSR     @DIVID
04052 151220            MOVZR   2,2
04053 112513            SKGT    0,2
04054 000403            JMP     .+3
04055 102520            SUBZL   0,0
04056 107000            ADD     0,1
04057 046252            STA     1,@STAPV
04060 020000            LDA     0,GCPT
04061 101005            SKNEZ   0,0
04062 000403            JMP     .+3
04063 006000            JSR     @APLOT
04064 000403            JMP     .+3
04065 046255            STA     1,@AVVAL
04066 010255            ISZ     AVVAL
04067 020137            LDA     0,SCNT
04070 042253            STA     0,@STOVC
04071 006302 SRCAL:     JSR     @TAPES
04072 060477            READS   0
04073 101122            MOVZL   0,0,SZC
04074 000424            JMP     SCOUT
04075 101122            MOVZL   0,0,SZC
04076 002421            JMP     @PREDY
04077 020136            LDA     0,NSCNS
04100 024137            LDA     1,SCNT
04101 106513            SKGT    0,1
04102 000416            JMP     SCOUT
04103 024247            LDA     1,DELAY
04104 125005            SKNEZ   1,1
04105 000407            JMP     .+7
04106 044000            STA     1,CNT
04107 060114            NIOS    RTC
04110 063614            SKPDN   RTC
04111 000777            JMP     .-1
```

METHOD II

Description of Program Modifications

The modifications made to SCAN commence at NLOOP. The peak counter (PCNT) is incremented and the current N-value (in AC-0) stored (NSTOR). SWTCH is then set equal to one and a jump made to S/R RDINT to collect the first (central) intensity. This intensity is checked to see if it is greater than zero, if not then it is put equal to zero. The intensity is then stored both in the B-buffer and in a temporary store (KEEP). If the intensity is greater than the threshold the quantitative section is entered, otherwise a jump is made to NL1 to continue the scan. In the quantitative section, SWTCH, a software flag for S/R RDINT (as used in method one), is set to one. A decremented N-value (NSTOR-DECRT) is then calculated and passed in AC-0 to S/R RDINT which passes back the second intensity in AC-0. This intensity is then stored in the B-buffer. An incremented N-value is then calculated, (NSTOR + DECRT), passed to S/R RDINT and the third intensity collected. These two latest intensities are then averaged by S/R DBLE, pointed to by PDBLE. The answer from this calculation (Intensity A) is then stored in HIDE. Another N-value (NSTORE - DECRT/2) is then calculated and passed to S/R RDINT. The fourth intensity collected is then stored in the B-buffer. Another correspondingly incremented N-value is calculated (NSTOR + DECRT/2) and passed to S/R RDINT. The fifth intensity is then passed back in AC-0. This is averaged with the fourth intensity in S/R DBLE. The resultant intensity B is then averaged with intensity A in S/R DBLE to give another intensity (Intensity C). This value is then averaged with the first intensity (from KEEP) to give a final value (Intensity D). Intensity D is then

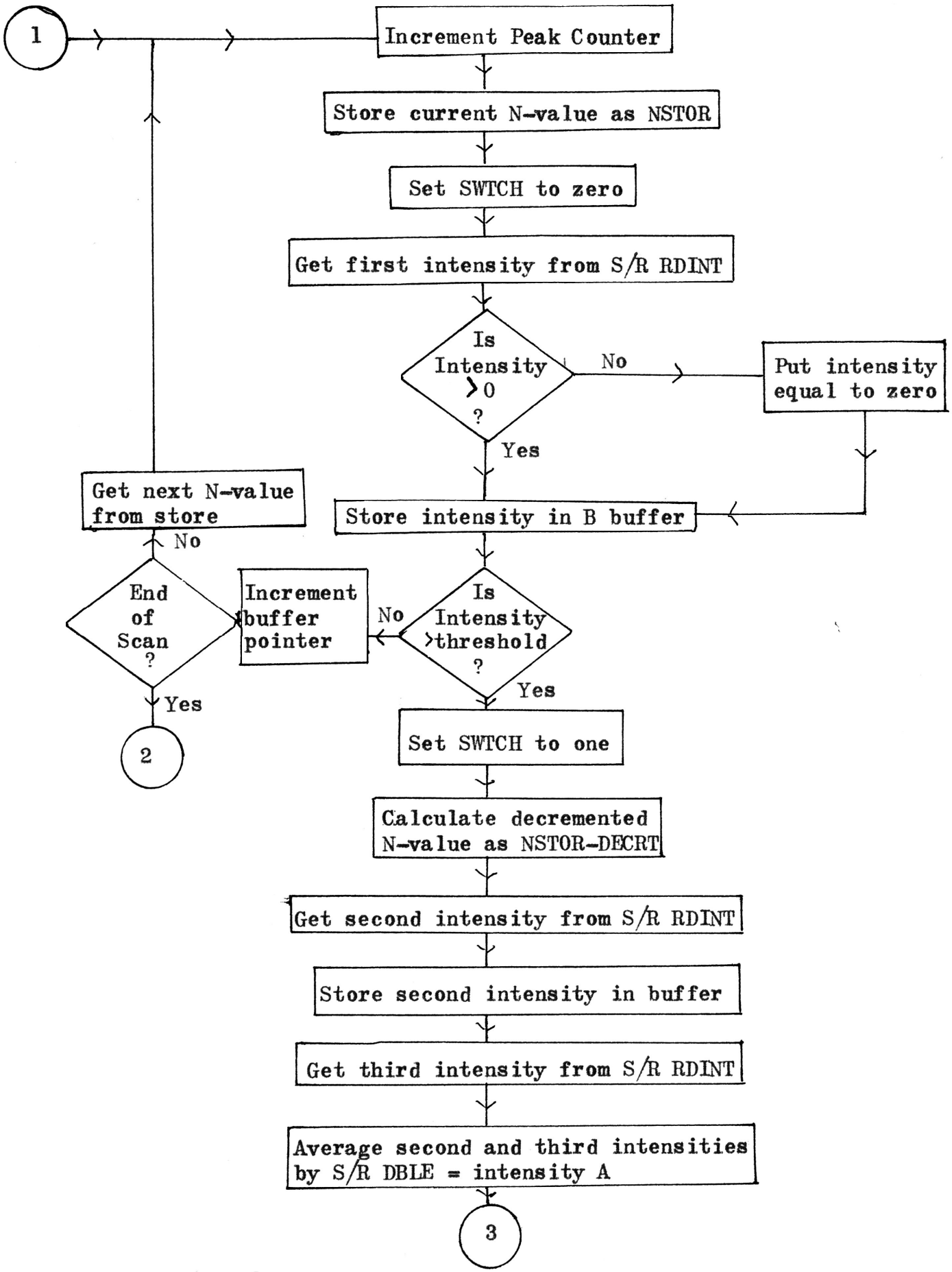
stored as the intensity at that mass value.

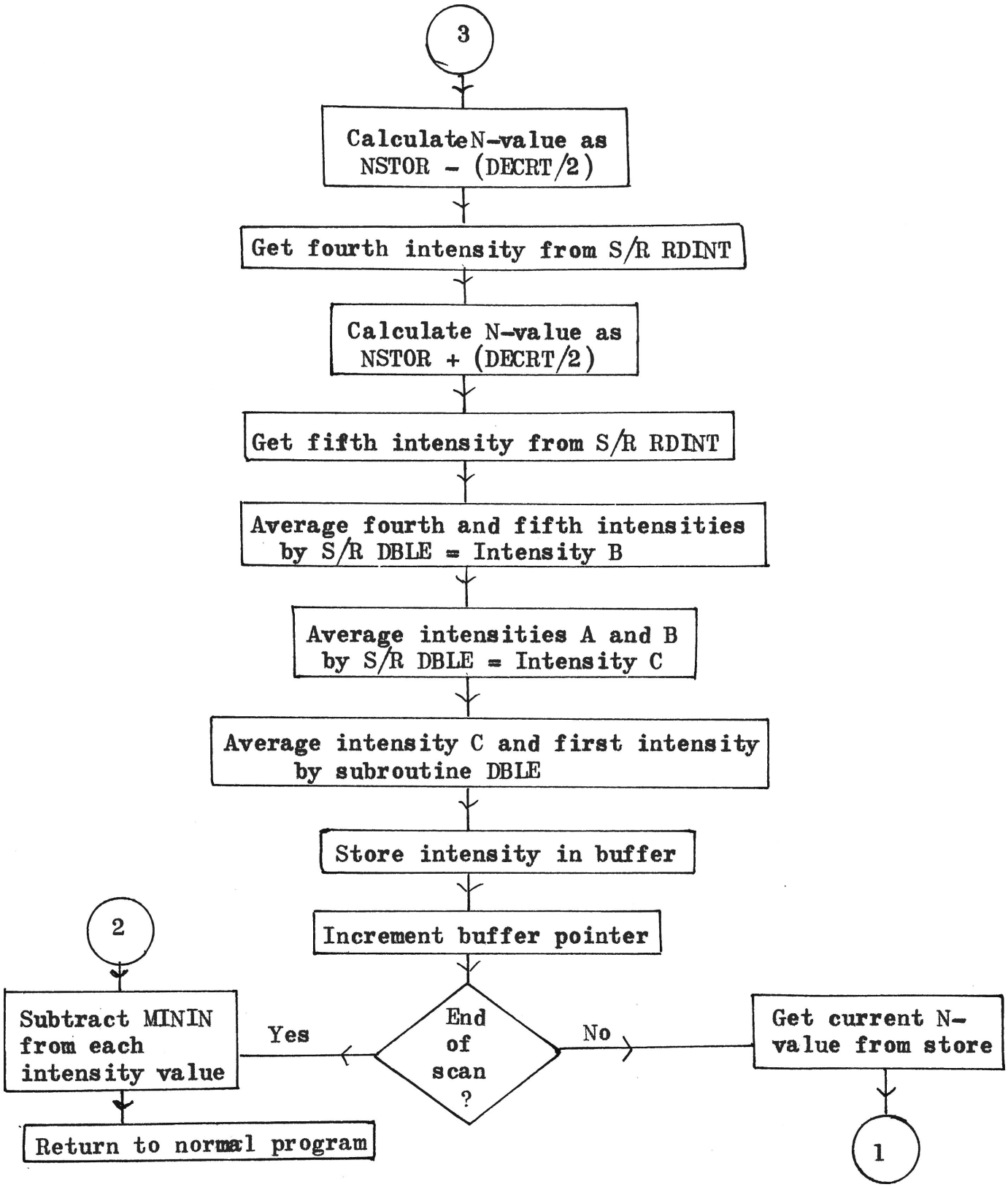
The intensity buffer pointer (ASET) is then incremented and PCNT, the number of peaks to be analysed and MCNT the current number of peaks examined compared. If MCNT is equal to PCNT then the scan is complete. If not then N-value previously calculated in RDINT is loaded into AC-0 and a jump made to NLOOP to continue the scan.

After termination of the scan the location of the first intensity value (BBUFF) is loaded into AC-2 and the number of intensity values loaded into a counter. A loop is then entered (NOISE) which subtracts MININ, the artificial noise level, from each intensity. The result is checked and if negative is replaced by zero. The total ion current is simulated by summing the intensities in this loop also (TOTIC). The corrected intensity is then stored back in the buffer and count checked to see if the spectrum has been run through. If so a jump is made to S/R SPZER to create a spectrum in the A-buffer compatible with that required by the DISPLAY segment. The rest of the scan segment is the same as that for the first method, including the change to S/R RDINT.

Subroutine DBLE is used to average the intensity values in this method. It is a self-contained subroutine which accepts two numbers, one in AC-0 and one stored in the intensity buffer, adds them together using the global subroutine, .DADD, (pointed to by DSUM) and divides the result by two. The intensity is then stored back in the intensity buffer and a normal subroutine return made.

FLOW CHART OF METHOD II (5 PEAKS)




3
Calculate N-value as NSTOR - (DECRT/2)
Get fourth intensity from S/R RDINT
Calculate N-value as NSTOR + (DECRT/2)
Get fifth intensity from S/R RDINT
Average fourth and fifth intensities by S/R DBLE = Intensity B
Average intensities A and B by S/R DBLE = Intensity C
Average intensity C and first intensity by subroutine DBLE
Store intensity in buffer
Increment buffer pointer
End of scan ?
Yes
No
2
Subtract MININ from each intensity value
Return to normal program
Get current N-value from store
1

```
---
↑↑↑
03747 010137  NSCAN:  ISZ     SCNT
03750 006266          JSR     @PABCL
03751 102440          SUBO    0,0
03752 040133          STA     0,OVCNT
03753 040113          STA     0,PCNT
03754 024227          LDA     1,CBUFF
03755 044226          STA     1,MPONT
03756 022226          LDA     0,@MPONT
03757 024232          LDA     1,BBUFF
03760 044231          STA     1,ASET
03761 006112          JSR     @MCONV
03762 010113  NLOOP:  ISZ     PCNT
03763 040551          STA     0,NSTOR
03764 126400          SUB     1,1
03765 044222          STA     1,SWTCH
03766 006114          JSR     @INTRD
03767 100513          SKGTZ   0,0
03770 121000          MOV     1,0
03771 042231          STA     0,@ASET
03772 040545          STA     0,KEEP
03773 024677          LDA     1,THRES
03774 106513          SKGT    0,1
03775 000436          JMP     NL1
03776 126520          SUBZL   1,1
03777 044222          STA     1,SWTCH
04000 024223          LDA     1,DECRT
04001 020533          LDA     0,NSTOR
04002 122400          SUB     1,0
04003 006114          JSR     @INTRD
04004 042231          STA     0,@ASET
04005 020527          LDA     0,NSTOR
04006 024223          LDA     1,DECRT
04007 123000          ADD     1,0
04010 006114          JSR     @INTRD
04011 006267          JSR     @PDBLE
04012 026231          LDA     1,@ASET
04013 044523          STA     1,HIDE
04014 024223          LDA     1,DECRT
04015 125220          MOVZR   1,1
04016 020516          LDA     0,NSTOR
04017 122400          SUB     1,0
04020 006114          JSR     @INTRD
04021 042231          STA     0,@ASET
04022 024223          LDA     1,DECRT
04023 125220          MOVZR   1,1
04024 123000          ADD     1,0
04025 006114          JSR     @INTRD
04026 006267          JSR     @PDBLE
04027 020507          LDA     0,HIDE
```

```
04030 006267            JSR     @PDBLE
04031 020506            LDA     0,KEEP
04032 006267            JSR     @PDBLE
04033 010231  NL1:      ISZ     ASET
04034 020113            LDA     0,PCNT
04035 024230            LDA     1,MCNT
04036 122513            SKGT    1,0
04037 000403            JMP     .+3
04040 020111            LDA     0,NVAL
04041 000721            JMP     NLOOP
04042 030232            LDA     2,BBUFF
04043 024113            LDA     1,PCNT
04044 044156            STA     1,CNT
04045 025000  NOISE:    LDA     1,0,2
04046 020677            LDA     0,MININ
04047 106400            SUB     0,1
04050 176400            SUB     3,3
04051 125112            SKGEZ   1,1
04052 165000            MOV     3,1
04053 045000            STA     1,0,2
04054 151400            INC     2,2
04055 102400            SUB     0,0
04056 006105            JSR     @DSUM
04057 000115            TOTIC
04060 040115            STA     0,TOTIC
04061 044116            STA     1,TOTIC+1
04062 014156            DSZ     CNT
04063 000762            JMP     NOISE
04064 006225            JSR     @PSPZR
04065 020115  SNCAL:    LDA     0,TOTIC
04066 024116            LDA     1,TOTIC+1
04067 030113            LDA     2,PCNT
04070 006123            JSR     @DIVID
04071 151220            MOVZR   2,2
04072 112513            SKGT    0,2
04073 000403            JMP     .+3
04074 102520            SUBZL   0,0
04075 107000            ADD     0,1
04076 046252            STA     1,@STAPV
04077 020256            LDA     0,GCPT
04100 101005            SKNEZ   0,0
04101 000403            JMP     .+3
04102 006261            JSR     @APLOT
04103 000403            JMP     .+3
04104 046255            STA     1,@AVVAL
04105 010255            ISZ     AVVAL
04106 020137            LDA     0,SCNT
04107 042253            STA     0,@STOVC
04110 006302  SRCAL:    JSR     @TAPES
04111 060477            READS   0
```

METHOD III

Description of Program Modifications

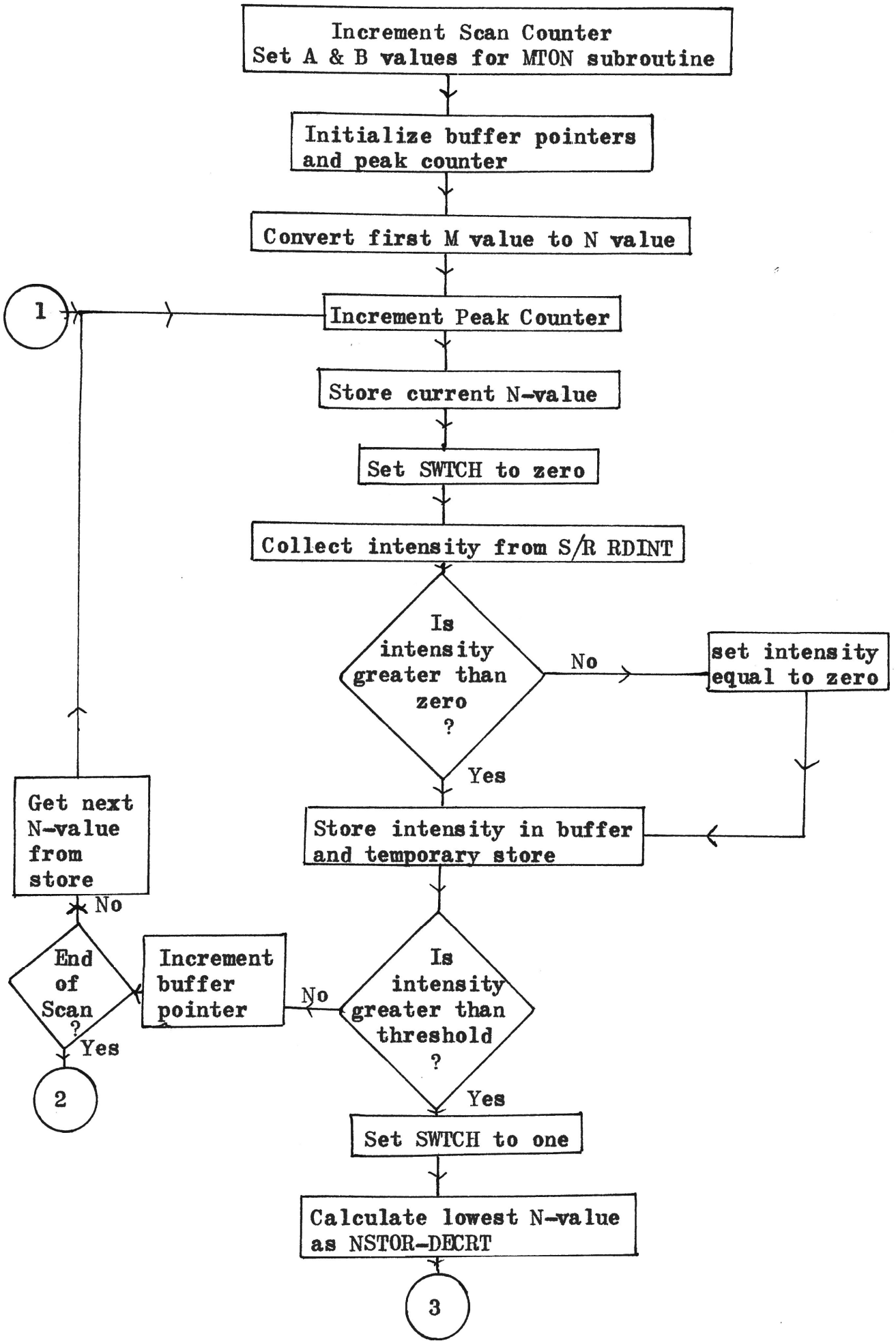
In general the early section of the scan segment is the same as for the other modifications. However a buffer to hold five intensities must be created to accommodate the intensities until the area is calculated. These five locations are called BUFFER and the pointer to their location is PIBUF. The modifications made start after NLOOP. The last N-value calculated by S/R RDINT and which would normally be used as the current N-value is stored in NSTOR. SWTCH is set to a value of zero and a jump made to S/R RDINT to collect the first N-value. This is checked to see if it is greater than zero, if not the intensity is put equal to zero. The intensity is then stored in the buffer and in the temporary store KEEP. A check is then made to see if the intensity is greater than the threshold (50% over intensity at m/e 287). If the threshold is not exceeded the program continues on to the collection of the intensity corresponding to the next selected mass number. If the threshold is equalled or exceeded SWTCH is set equal to unity. The decremented N-value is then calculated by taking the normal N-value and subtracting from it a preset integer decrement. This then becomes the lowest N-value of the five for the peak area calculation and is stored at the locations. FIRSN and HIDE. The number of intensities to be collected (5) is loaded from its storage location (CON5) into a pass counter (PASS). The location of the five membered buffer to temporarily store the intensity values is loaded into a pointer (PIBUF). A loop is then entered (NL2) which collects and stores the intensity values. The N-value to be used is collected from temporary store (HIDE) and compared

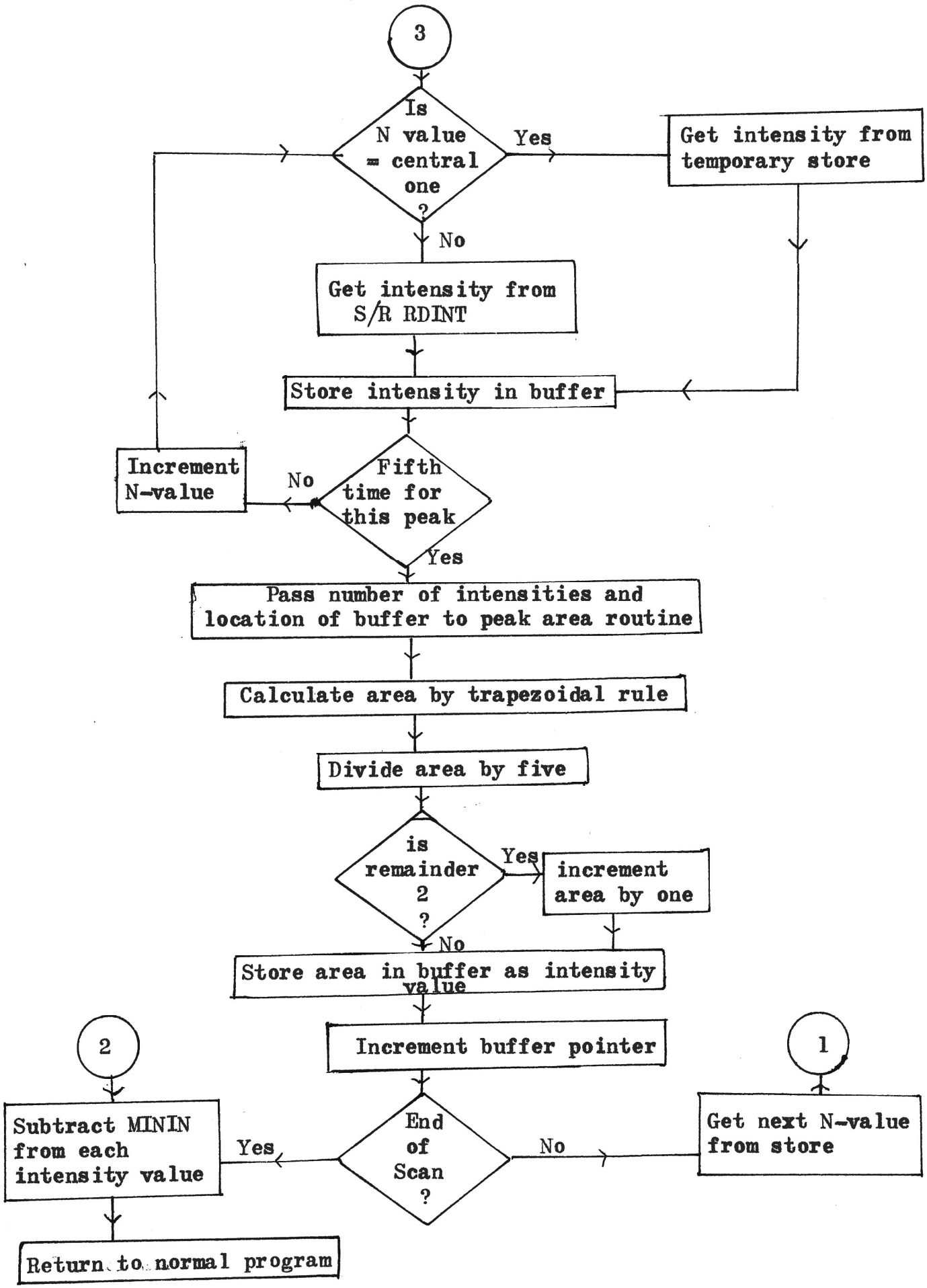
to the central N-value. If the current N-value is the same as the central N-value, whose intensity has already been collected, the program retrieves the intensity from its temporary store (KEEP). Otherwise the subroutine RDINT is entered to collect another intensity at the N-value supplied. On return the intensity collected (whether it be from RDINT or from KEEP) is stored in the buffer pointed to by PIBUF. PIBUF and HIDE (the current N-value) are then incremented and the loop counter (PASS) examined to see if five intensities have been collected. If so a jump is made out of the loop, otherwise a jump is made to NL2 to collect the next intensity value. At completion of the loop the data needed to calculate the area is set up in the appropriate accumulators and locations. Since the area subroutine uses hardware Accumulator 2 and floating point accumulators 1 and 2, these must be stored since they contain data vital for the M to N conversion subroutine. The number of intensity values is passed in Accumulator 0, the lowest N-value in Accumulator 1 and the address of the buffer is pointed to by Accumulator 2. The subroutine to calculate the peak area by the trapezoidal rule is pointed to by location 337. The location of the resultant area is pointed to by location 376 and the answer is stored as a double precision number, high order followed by low order. This number is then divided by the number of intensity values (5) by means of subroutine DIVID. For this subroutine the divident is passed in AC-0 (high order) and AC-1 (low order) and the divisor in AC-2. The quotient is returned in AC-1 and the remainder in AC-0. This remainder is checked and if greater than two (i.e. since it is an integer it will be greater than half of five) the quotient is incremented by one to "round up". The quotient is then

stored as an intensity at the location pointed to by ASET. AC-2 and floating point AC-1 and AC-2 are then restored to enable M to N conversion to occur in subroutine RDINT. The intensity buffer pointer (ASET) is then incremented and PCNT (the number of peaks) compared to MCNT (the running total of peaks analysed) to see if the scan is completed. If the scan is completed an exit is made, otherwise the N-value previously calculated in S/R RDINT is loaded into AC-0 and a jump made to NLOOP to collect the intensity for the next desired mass value.

After termination of the scan the location of the first intensity value (BBUFF) is loaded into accumulator 2 and the number of peaks into a counter (CNT). A loop is then entered (NOISE) which subtracts MININ from each intensity value, checks if it is negative and if so sets equal to zero, stores the intensity back in the B-buffer and calculates the total intensity for the spectrum. After exit from this loop a jump is made to subroutine SPZER which creates a spectrum in the A-buffer which is consistent with the needs of the Display segment.

FLOW CHART OF METHOD III




3
Is N value = central one ?
Yes
Get intensity from temporary store
No
Get intensity from S/R RDINT
Store intensity in buffer
Increment N-value
No
Fifth time for this peak
Yes
Pass number of intensities and location of buffer to peak area routine
Calculate area by trapezoidal rule
Divide area by five
is remainder 2 ?
Yes
increment area by one
No
Store area in buffer as intensity value
Increment buffer pointer
2
1
Subtract MININ from each intensity value
Yes
End of Scan ?
No
Get next N-value from store
Return to normal program

```
---
↑↑↑
03770 010137  NSCAN:  ISZ     SCNT
03771 006266          JSR     @PABCL
03772 102440          SUBO    0,0
03773 040133          STA     0,OVCNT
03774 040113          STA     0,PCNT
03775 024227          LDA     1,CBUFF
03776 044226          STA     1,MPONT
03777 022226          LDA     0,@MPONT
04000 024232          LDA     1,BBUFF
04001 044231          STA     1,ASET
04002 006112          JSR     @MCONV
04003 010113  NLOOP:  ISZ     PCNT
04004 040573          STA     0,NSTOR
04005 126400          SUB     1,1
04006 044222          STA     1,SWTCH
04007 006114          JSR     @INTRD
04010 126400          SUB     1,1
04011 100513          SKGTZ   0,0
04012 121000          MOV     1,0
04013 042231          STA     0,@ASET
04014 040735          STA     0,KEEP
04015 024655          LDA     1,THREE
04016 106513          SKGT    0,1
04017 000457          JMP     NL1
04020 126520          SUBZL   1,1
04021 044222          STA     1,SWTCH
04022 024223          LDA     1,DECRT
04023 020554          LDA     0,NSTOR
04024 122400          SUB     1,0
04025 040723          STA     0,HIDE
04026 040720          STA     0,FIRSN
04027 024724          LDA     1,CON5
04030 044717          STA     1,PASS
04031 034723          LDA     3,BUFER
04032 054720          STA     3,PIBUF
04033 024544  NL2:    LDA     1,NSTOR
04034 020714          LDA     0,HIDE
04035 106414          SKEQ    0,1
04036 000403          JMP     .+3
04037 020712          LDA     0,KEEP
04040 000402          JMP     .+2
04041 006114          JSR     @INTRD
04042 042710          STA     0,@PIBUF
04043 010707          ISZ     PIBUF
04044 010704          ISZ     HIDE
04045 014702          DSZ     PASS
04046 000765          JMP     NL2
04047 024704          LDA     1,CON5
04050 020676          LDA     0,FIRSN
```

```
04051 006004            FETR
04052 044711            FSTA    1,FSTOR
04053 050712            FSTA    2,FSTOR+2
04054 100000            FEXT
04055 050705            STA     2,TWOST
04056 030676            LDA     2,BUFER
04057 006337            JSR     @PKARR
04060 030376            LDA     2,PAREA
04061 021000            LDA     0,0,2
04062 025001            LDA     1,1,2
04063 030670            LDA     2,CON5
04064 006123            JSR     @DIVID
04065 151220            MOVZR   2,2
04066 112512            SKLE    0,2
04067 125400            INC     1,1
04070 046231            STA     1,@ASET
04071 030671            LDA     2,TWOST
04072 006004            FETR
04073 024670            FLDA    1,FSTOR
04074 030671            FLDA    2,FSTOR+2
04075 100000            FEXT
04076 010231  NL1:      ISZ     ASET
04077 020113            LDA     0,PCNT
04100 024230            LDA     1,MCNT
04101 122513            SKGT    1,0
04102 000403            JMP     .+3
04103 020111            LDA     0,NVAL
04104 000677            JMP     NLOOP
04105 030232            LDA     2,BBUFF
04106 024113            LDA     1,PCNT
04107 044156            STA     1,CNT
04110 025000  NOISE:    LDA     1,0,2
04111 020634            LDA     0,MININ
04112 106400            SUB     0,1
04113 176400            SUB     3,3
04114 125112            SKGEZ   1,1
04115 165000            MOV     3,1
04116 045000            STA     1,0,2
04117 151400            INC     2,2
04120 102400            SUB     0,0
04121 006105            JSR     @DSUM
04122 000115            TOTIC
04123 040115            STA     0,TOTIC
04124 044116            STA     1,TOTIC+1
04125 014156            DSZ     CNT
04126 000762            JMP     NOISE
04127 006225            JSR     @PSPZR
04130 020115  SNCAL:    LDA     0,TOTIC
04131 024116            LDA     1,TOTIC+1
04132 030113            LDA     2,PCNT
```

Subsidiary Modifications

Subroutine QANTM

This subroutine begins by storing the address for normal subroutine return. Before the subroutine call a message "MASS VALUES" was typed. A message is now written (SMES1) and instructs the user to "ENTER LIST". A counter (MCNT) is initialised to zero and a carriage return and line feed command sent to the teletype. Global subroutine DATIN is then called to collect a number and its break character from the teletype. If an erroneous response is received by S/R DATIN a JMP @ GOBAK occurs and the program is re-entered before the jump to the subroutine QANTM. If a correct response is received the mass value is stored in the mass buffer (top section of the B-buffer). The mass number is checked to see if it is within the acceptable range (less than 700) and if not a jump to subroutine EXIT is made. The number of mass numbers collected so far is then checked and if equal to or greater than 325 a jump to S/R EXIT is made to terminate the entry of mass numbers. The break character is then examined and if equal to a carriage return (the indication that the entry of mass numbers has been completed) then a jump is made to S/R EXIT. The break character, if not a carriage return, is then examined to see if it is a dash ("-"). A dash between mass numbers is meant to indicate that a range of mass numbers is desired to be scanned and so a second mass number must be collected. If the break character is not a dash then it is examined to see if it is a semi-colon (an indication that another line is necessary for entry of mass numbers) and if so a carriage return and line feed commands are sent to the teletype. If the break

character is none of these characters then it is assumed that the user wishes to enter another mass value and the program returns to INN to accept another mass value.

If the break character was a dash as mentioned above then a range of mass values is desired. The first mass value is stored as MSTAR, the initial mass value of the range. The final mass value is then collected by the use of S/R DATIN. The second break character is then stored and the mass value examined to see that it falls within the acceptable mass limit (700 a.m.u), if not it is replaced by 700. The second mass number is then stored as MSTOP the final mass value of the range. The initial mass value is then incremented, stored in the M-buffer and the buffer pointer and the mass counter incremented. The mass counter is checked against the limit constrained by half the B-buffer (325) and if greater than or equal to the limit a jump made to S/R EXIT. Otherwise the mass value is checked against the MSTOP value to see if the range has been covered by placing the mass values in the M-buffer. If not the loop is entered again to increment the mass value and store it in the M-buffer. If the range is complete the break character of the second (MSTOP) mass value collected is examined to see if it is a semi-colon. If so a carriage return and line feed are sent to the teletype before a new mass value is collected by a jump to INN. If the break character is a carriage return accumulator one is loaded with MSTOP and a jump made to EXIT + 1. If the break character is neither of these two then a jump to INN is made to collect another mass value.

Subroutine EXIT is simply a handy technique for performing all the storing of initial and final mass values in one place. The MSTAR and

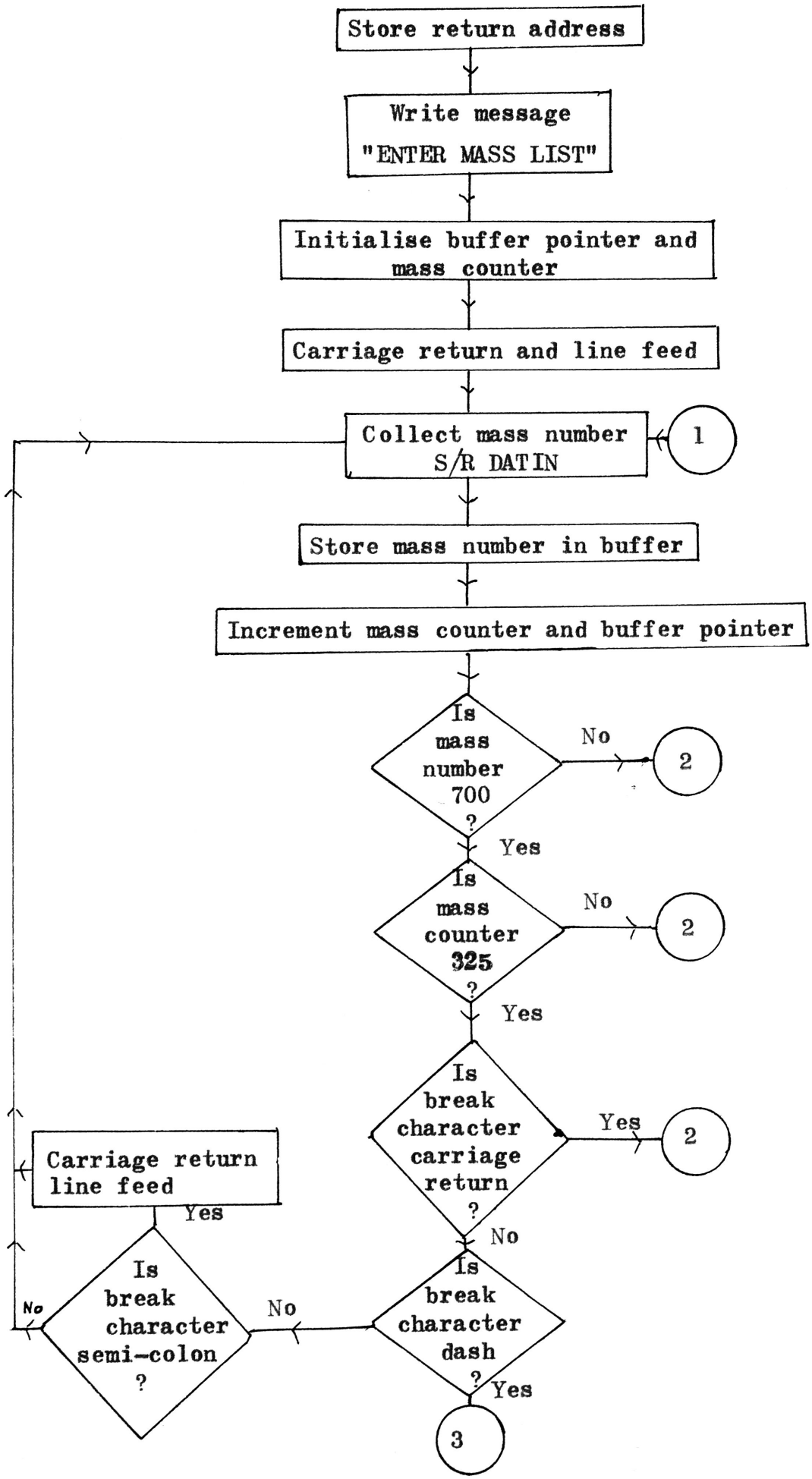
MSTOP values are stored in the B-buffer and in page zero with EXIT always being entered with AC-1 containing MSTOP. The subroutine returns to the address after the call for subroutine QANTM.

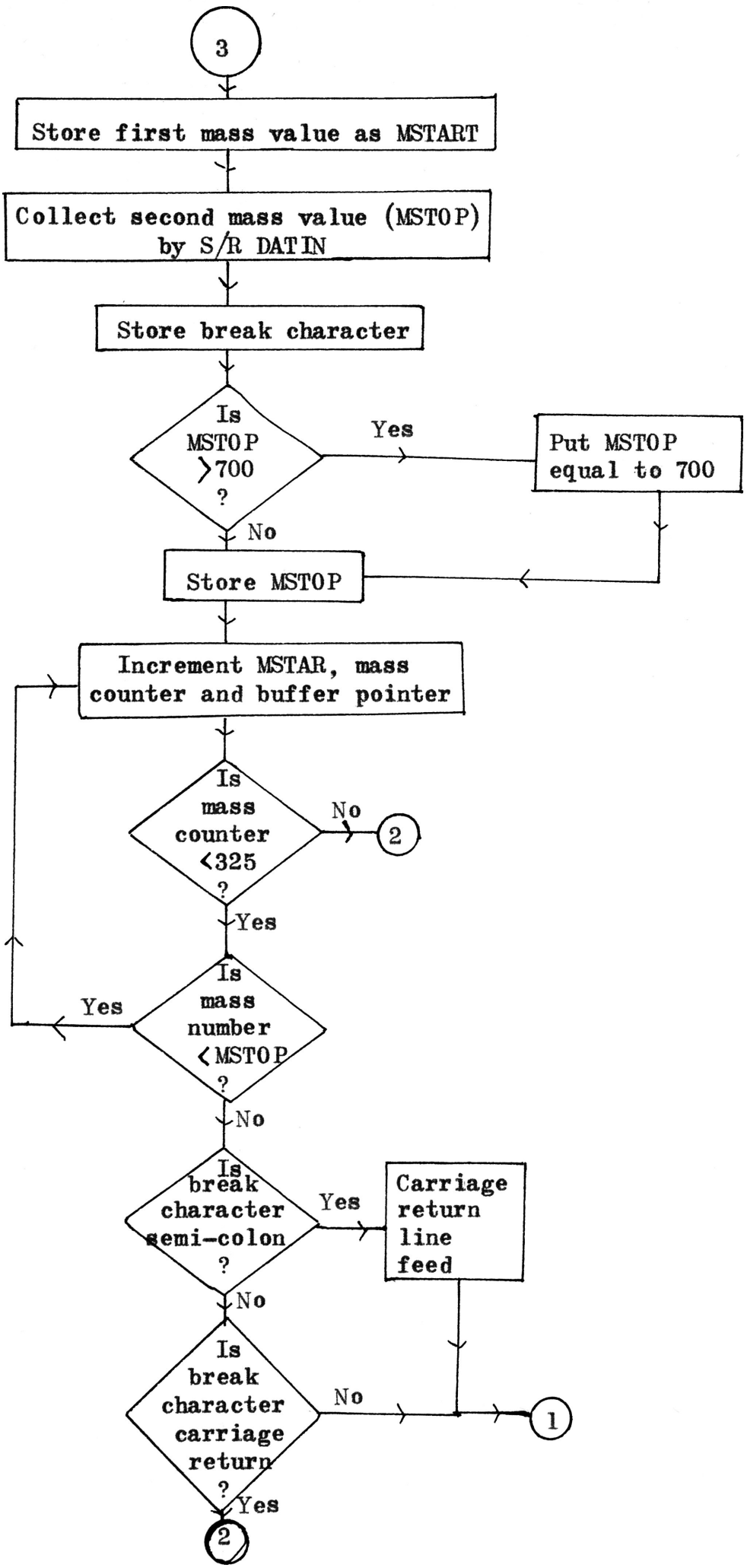
Subroutine SPZER

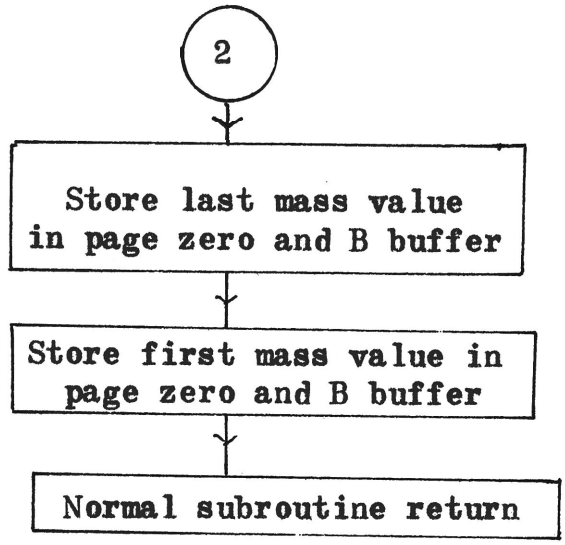
This subroutine is associated with subroutine QANTM and creates an intensity spectrum in the A-buffer compatible with DISPLAY.

The subroutine commences by storing its return address at SPRET. The number of mass peaks scanned is then loaded into a counter (MPCNT) and pointers to the A-buffer (ASET), the B-buffer (BSET) and the M-buffer (MSET) initialised. The first mass value is then loaded into AC-0 and AC-2. AC-1 is loaded with the first intensity value and AC-3 set to zero. At SPECT AC-1 is stored in the A-buffer. The counter is decremented and if zero a normal subroutine return made since the spectrum would have been completely transferred to the A-buffer. If the counter is non-zero then AC-0 is loaded with the next M-value from the M-buffer, the B-buffer pointer and the M-buffer pointer are incremented as is the contents of AC-2, the current mass value. The A-buffer pointer is then incremented and accumulators two and zero compared. AC-2 contains the incremented mass value and AC-0 the next mass value from the buffer so that if a jump of mass values has been specified a difference will exist between AC-0 and AC-2. If so then the contents of AC-3 are stored as intensity (zero value) in the A-buffer until the mass values coincide when a jump is made to SPECT to continue the process with another mass value from the M-buffer.

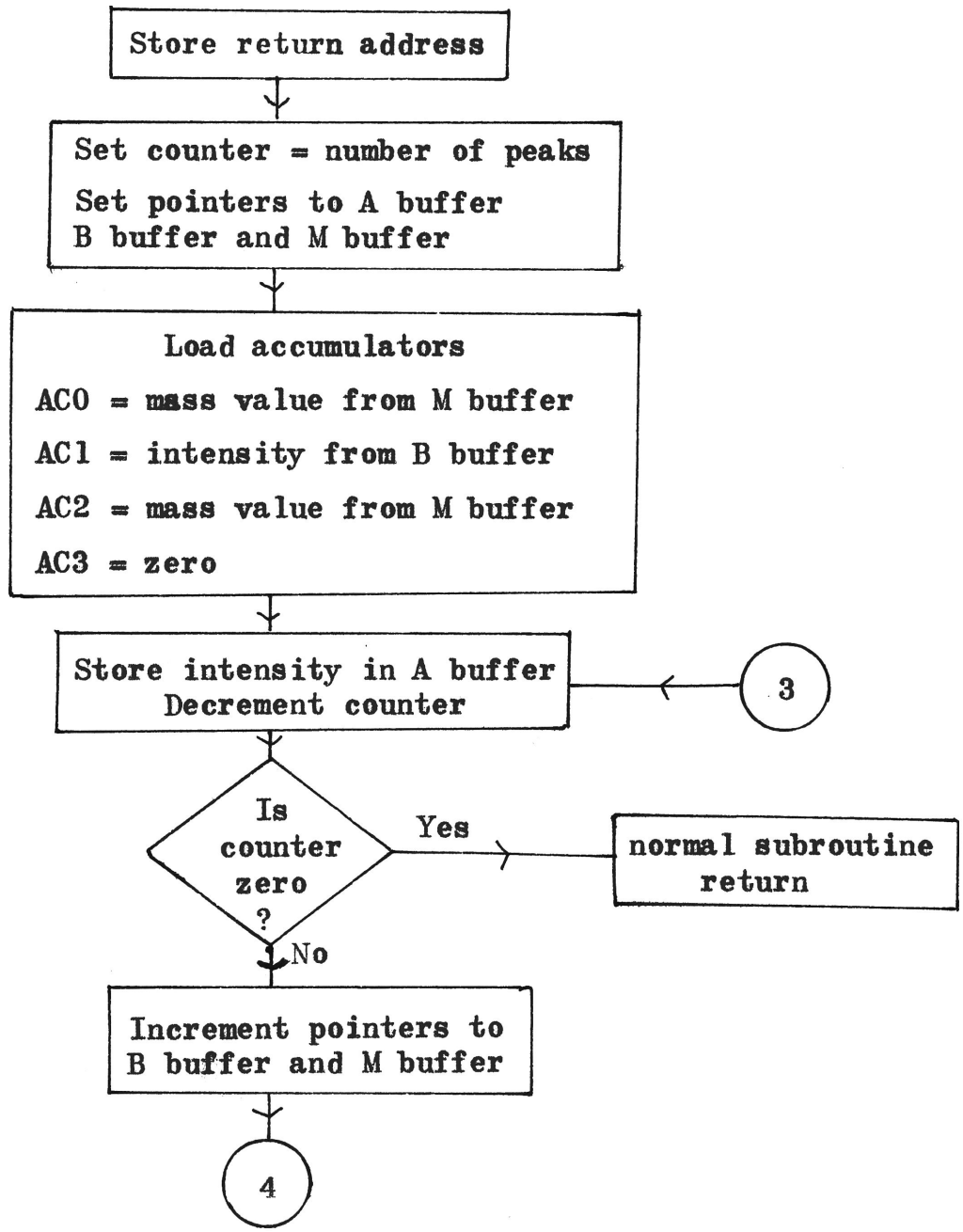
FLOW CHART OF SUBROUTINE QANTM

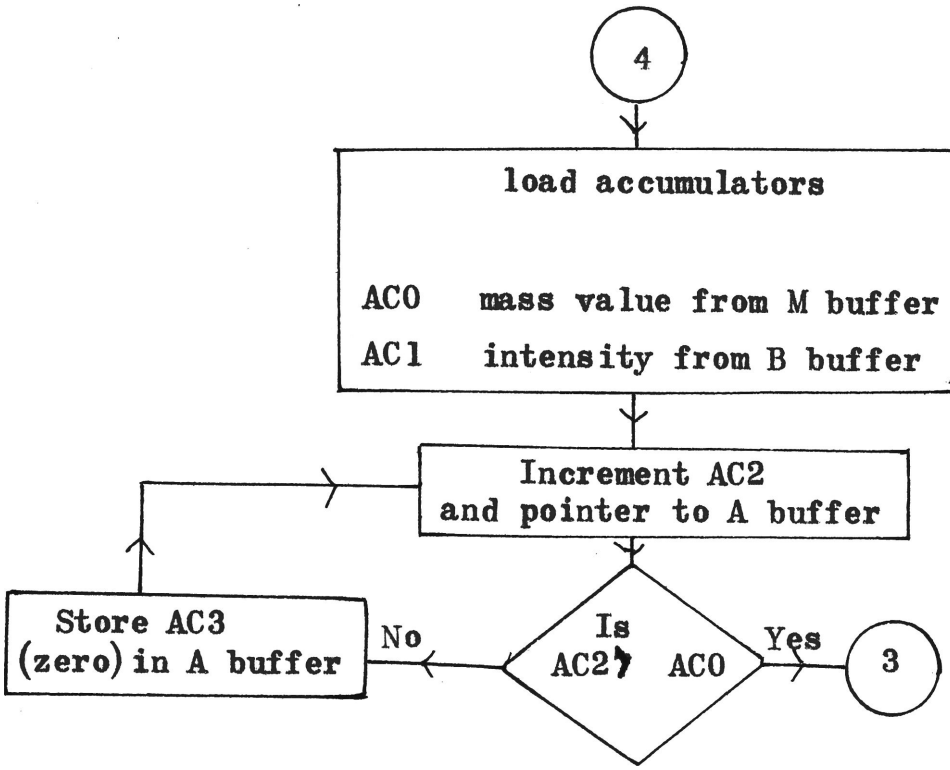



3
Store first mass value as MSTART
Collect second mass value (MSTOP) by S/R DATIN
Store break character
Is MSTOP >700 ?
Yes
Put MSTOP equal to 700
No
Store MSTOP
Increment MSTAR, mass counter and buffer pointer
Is mass counter <325 ?
No
2
Yes
Is mass number <MSTOP ?
Yes
No
Is break character semi-colon ?
Yes
Carriage return line feed
No
Is break character carriage return ?
No
1
Yes
2



FLOW CHART SUBROUTINE SPZER




4
load accumulators
AC0 mass value from M buffer
AC1 intensity from B buffer
Increment AC2
and pointer to A buffer
Store AC3
(zero) in A buffer
No
Is
AC2 ? AC0
Yes
3

```
04300 054473  QANTM:  STA     3,MRET
04301 030500          LDA     2,SMES1
04302 006044          WRITE
04303 034227          LDA     3,CBUFF
04304 054226          STA     3,MBUFF
04305 102400          SUB     0,0
04306 040230          STA     0,MCNT
04307 006052          CARLF
04310 006131  INN:    JSR     @DATIN
04311 002132          JMP     @GOBAK
04312 046226          STA     1,@MBUFF
04313 010226          ISZ     MBUFF
04314 010230          ISZ     MCNT
04315 034457          LDA     3,MRLIM
04316 136512          SKLE    1,3
04317 000471          JMP     EXIT
04320 034460          LDA     3,MLIM
04321 030230          LDA     2,MCNT
04322 156512          SKLE    2,3
04323 000465          JMP     EXIT
04324 034060          LDA     3,CARRT
04325 162415          SKNEQ   3,0
04326 000462          JMP     EXIT
04327 034446          LDA     3,CDSH
04330 162415          SKNEQ   3,0
04331 000405          JMP     .+5
04332 034444          LDA     3,SEMIC
04333 116415          SKNEQ   0,3
04334 006052          CARLF
04335 000753          JMP     INN
04336 044001          STA     1,MSTAR
04337 006131          JSR     @DATIN
04340 002132          JMP     @GOBAK
04341 040436          STA     0,BRKCH
04342 034432          LDA     3,MRLIM
04343 166513          SKGT    3,1
04344 165000          MOV     3,1
04345 044002          STA     1,MSTOP
04346 024001          LDA     1,MSTAR
04347 125400          INC     1,1
04350 046226          STA     1,@MBUFF
04351 010226          ISZ     MBUFF
04352 010230          ISZ     MCNT
04353 020425          LDA     0,MLIM
04354 034230          LDA     3,MCNT
04355 116513          SKGT    0,3
04356 000432          JMP     EXIT
04357 030002          LDA     2,MSTOP
04360 146512          SKLE    2,1
```

```
---
04361 000766            JMP     .-12
04362 020415            LDA     0,BRKCH
04363 024413            LDA     1,SEMIC
04364 106415            SKNEQ   0,1
04365 000722            JMP     INN-1
04366 024060            LDA     1,CARRT
04367 106414            SKEQ    0,1
04370 000720            JMP     INN
04371 024002            LDA     1,MSTOP
04372 000417            JMP     EXIT+1
04373 000000  MRET:     0
04374 001274  MRLIM:    700.
      000060  CARRT=60
04375 000055  CDSH:"-
04376 000073  SEMIC:    ";
04377 000000  BRKCH:    0
04400 000505  MLIM:     325.
04401 004402  SMES1:    .+1
04402 047105            .TXT*EN
04403 042524  TE
04404 020122  R
04405 044514  LI
04406 052123  ST
04407 000000  *

04410 044002  EXIT:     STA     1,MSTOP
04411 046251            STA     1,@STOPM
04412 036227            LDA     3,@CBUFF
04413 054001            STA     3,MSTART
04414 056250            STA     3,@STARM
04415 002756            JMP     @MRET
04416 054435  SPZER:    STA     3,SPRET
04417 020113            LDA     0,PCNT
04420 040431            STA     0,MPCNT
04421 020000            LDA     0,ABUFF
04422 040231            STA     0,ASET
04423 020232            LDA     0,BBUFF
04424 040426            STA     0,BSET
04425 020227            LDA     0,CBUFF
04426 040226            STA     0,MBUFF
04427 022226            LDA     0,@MBUFF
04430 111000            MOV     0,2
04431 026421            LDA     1,@BSET
04432 176400            SUB     3,3
04433 046231  SPECT:    STA     1,@ASET
04434 014415            DSZ     MPCNT
04435 000402            JMP     .+2
04436 002415            JMP     @SPRET
04437 010413            ISZ     BSET
04440 010226            ISZ     MBUFF
```

```
04441 022226            LDA     0,@MBUFF
04442 026410            LDA     1,@BSET
04443 151400            INC     2,2
04444 010231            ISZ     ASET
04445 142415            SKNEQ   2,0
04446 000765            JMP     SPECT
04447 056231            STA     3,@ASET
04450 000773            JMP     .-5
04451 000000  MPCNT:    0
04452 000000  BSET:     0
04453 000000  SPRET:    0
                        .END
```

BIBLIOGRAPHY

1. R. M. Caprioli, W. F. Fies and M. S. Story, Analytical Chemistry, 46(4), 453A, (1974).

2. P. S. Baker, Survey of Progress in Chemistry, 4, 69, (1968).

3. W. F. Holmes, W. H. Holland, B. L. Shore, D. M. Bier and W. R. Sherman, Anal. Chem., 45(12), 2063, (1973).

4. P. D. Klein, J. R. Haumann and W. J. Eisler, Anal. Chem., 44(3), 490, (1972).

5. R. M. Caprioli, Finnigan Spectra, 3(3), 1, (1973).

6. J. H. Beynon, J. E. Corn, W. E. Baitinger, J. W. Amy and R. A. Benkser, Organic Mass Spectrometry, 3, 191, (1970).

7. G. E. Calf and J. L. Garnett, J. Phys. Chem., 68, 3387, (1964).

8. G. E. Calf, Ph.D thesis, University of New South Wales, 1966.

9. R. J. Harper and C. Kemball, J. Chem. Soc., 2224, (1969).

10. S. Olsson, Arkiv fur Kemi, 32(2), 105, (1970).

11. W. E. Weening, Chemisch Weekblad, 62, 153, (1966).

12. I. F. Tupitsyn and N. N. Zaptsepina, Isotopenpraxis, 3(7), 269, (1967).

13. R. R. Fraser and R. N. Renaud, J. Am. Chem. Soc., 88(19), 4365, (1966).

14. J. L. Garnett, Proceedings of the Second International Conference on methods of preparing and storing labelled compounds, Brussels, (1966).

15. J. L. Garnett, Catalysis Reviews, 5(2), 229, (1971).

16. J. L. Garnett and W. A. Sollich, Aust. J. Chem., 14, 441, (1961).

17. R. C. Murphy, Finnigan Spectra, 3(3), 4, (1973).

18. J. Horitui and M. Polanyi, Nature, 132, 819, (1933).

19. A. Farkas and L. Farkas, Proc. Royal Soc. (London), A144, 467 (1934).

20. T. I. Taylor, in Catalysis (P. H. Emmett editor), Vol. V, Reinhold, New York, 1957.

21. J. L. Garnett, Proc. Royal Australian Chem. Inst., 28(8), 328, (1961).

22. G. C. Bond, Catalysis by Metals, Academic Press, New York, (1962).

23. E. Crawford and C. Kemball, Trans. Faraday Soc., 58, 2452, (1962).

24. J. J. Rooney, J. Catalysis, 2, 52, (1963).

25. R. S. Mulliken, J. Am. Chem. Soc., 74, 811, (1952).

26. J. L. Garnett and W. A. Sollich-Baumgartner, Advances in Catalysis, 16, 95, (1966).

27. J. C. P. Mignolet, "Chemisorption", (W. E. Garner Editor), Academic Press, New York, (1957).

28. P. W. Selwood, J. Am. Chem. Soc., 79, 3346, 4537, 5391, (1957).

29. G. C. Macdonald and J. S. Shannon, Aust. J. Chem., 18, 1009, (1965).

30. C. Kemball, in Proceedings of the Fourth International Congress of Catalysis, Moscow, (1968).

31. J. L. Garnett and W. A. Sollich-Baumgartner, J. Catalysis, 5, 244, (1966).

32. W. K. Hannan, personal communication.

33. W. G. Brown and J. L. Garnett, J. Am. Chem. Soc., 80, 5272, (1958).

34. R. M. Keefer and L. T. Andrews, J. Am. Chem. Soc., 72, 4677, 5034, 5170, (1950).

35. F. A. Matson, A. C. Makrides and N. Hackerman, J. Chem. Phys., 22, 1800, (1965).

36. J. L. Garnett, L. J. Henderson, W. A. Sollich and G. V. D. Tiers, Tetrahedron Letters, 15, 516 (1961).

37. J. S. Shannon, Proc. Royal Australian Chem. Inst., 36(7), 185, (1969).

38. F. G. Gault, J. J. Rooney and C. Kemball, J. Catalysis, 1, 255, (1962).

39. K. Hirota and T. Ueda, Proc. Third International Congress on Catalysis, Amsterdam, (1964).

40. J. L. Garnett and W. A. Sollich-Baumgartner, J. Phys. Chem., 69, 1850, (1965).

41. J. L. Garnett, L. Henderson and W. A. Sollich, "Tritium in the Physical and Biological Sciences", Vol.II, 47, I.A.E.A., Vienna, (1962).

42. K. E. Wilzbach, reference 41, page 3.

43. M. Wenzel, H. Wallenberg and P. E. Schulze, reference 41, page 37.

44. G. E. Calf and J. L. Garnett, Chem. Commun., 373, (1969).

45. J. Graff and D. Ritenberg, Anal. Chem., 24(5), 878, (1952).

46. J. L. Garnett and W. A. Sollich, Aust. J. Chem., 15(1), 56, (1962).

47. D. H. Wiffen and H. W. Thompson, J. Chem. Soc., 268, (1945).

48. A. R. H. Cole and H. W. Thompson, Trans. Faraday Soc., 46(2), 103, (1949).

49. R. P. Bell, H. W. Thompson and E. E. Vago, Proc. Roy. Soc. (London), 192A, 498, (1948).

50. G. V. D. Tiers and J. H. Tiers, J. Chem. Physics, 120, 761, (1952).

51. G.V. D. Tiers, J. Chem. Phys., 29, 963, (1963).

52. C. C. Hinckley, J. Am. Chem. Soc., 91, 5160, (1969).

53. B. C. Mayo, Chemical Society Reviews, 2(1), 49, (1973).

54. J. E. Maskasky and M. E. Kenney, J. Am. Chem. Soc., 95(5), 1443, (1973).

55. J. M. Sanders and D. H. Williams, J.C.S. Chem. Commun., 436, (1972).

56. C. C. Hinckley, G. C. Smith and W. A. Boyd, J. Am. Chem. Soc. 93(23), 6319, (1971).

57. J. M. Sanders and D. H. Williams, J. Am. Chem. Soc., 93(3), 641 (1971).

58. J. P. Shoeffner, J. Am. Chem. Soc., 96(5), 1599, (1974).

59. A. I. Vogel, A Textbook of Practical Organic Chemistry, including qualitative organic analysis, 3rd ed., Longmans, London (1956).

60. "Handbook of Chemistry and Physics", 53rd edition, Chemical Rubber Co., 1973.

61. A. O. Nier, Phys. Rev., 79, 450, (1950).

62. C. E. Melton, W. Massey and B. N. Abels, Z. Naturforsch, 26a, 1241, (1971).

63. K. Bruchhausen, S. Gebauer and U. Von Zahn, Z. Naturforsh, 22a, 969, (1967).

64. G. B. Bunyard, The U.Ti Journal, 1(2), 1, (1973).

65. I. Miller and J. E. Freund, Probability for Statistics and Engineers, Prentice-Hall, New Jersey, (1965).

66. G. E. Ericksen, Scientific Enquiry in the Behavioural Sciences, Scott, Foresman and Company, Illinois, (1970).

67. R. M. Caprioli, Finnigan Spectra, 4(2), 3, (1974).

ACKNOWLEDGEMENTS

The author would like to express his gratitude to Mr. K. W. Hannan for proposing the outline of this project and his expert guidance and assistance during the year.

Thanks are also due to Associate Professor P. D. Bolton for his advice concerning the Quadnova modifications, to J. Korth for assistance with the recording of mass spectra and to the staff and research students of the department generally.

Finally the author acknowledges the encouragement given by his parents throughout his studies.